Behavioural and pharmacological characterization of a mouse model for psychotic disorders – focus on glutamatergic transmission

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ACADEMIC DISSERTATION

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ABSTRACT

Bipolar disorder, schizophrenia and schizoaffective disorder are extremely debilitating illnesses that encompass affective and/or psychotic symptoms. Not only is there common symptomatology and genetic susceptibility, but the pharmacotherapy approaches are also similar. Nonetheless, molecular mechanisms underpinning these diseases are not yet fully understood. The theory that there is a dopaminergic dysfunction cannot account for all of the symptoms. Nor can the compounds that act on dopaminergic mechanisms successfully alleviate the symptoms. There is evidence to suggest that there are imbalances in other neurotransmitter systems, particularly the main excitatory pathway - the glutamatergic system. Glutamatergic transmission is essential for development, learning and memory and many other physiological functions of the brain. Glutamatergic receptors of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type mediate the majority of the fast synaptic neurotransmission. Here, *Gria1−/−* mice, lacking the GluA1 subunit of AMPA receptors, with concurrent schizophrenia-like and affective symptoms were used. The predictive validity was addressed using the standard and novel glutamate-modulating pharmacotherapeutics. The hyperactivity, the most robust feature of *Gria1−/−* mice and a hallmark of psychotic disorders, was attenuated by drug-treatments. Importantly, chronic treatments with lithium, valproate, topiramate, lamotrigine and perampanel were effective, evidence of their pharmacological efficacy after the acute, often sedative, treatment phase. In addition, excessive novelty-induced activation of the dorsal hippocampus of *Gria1−/−* mice as measured by c-Fos expression was blunted by the drug-treatments of which all are known to reduce the activity of the glutamatergic transmission. Other behaviours relevant to the schizoaffective symptomatology such as disinhibited risk-taking, less despair-like behaviour and highly exploratory phenotype as well as social deficits were partially responsive to treatment with mood-stabilisers. Moreover, *Gria1−/−* mice exhibited a slightly higher preference for sucrose and made more impulsive choices towards sucrose. The *Gria1−/−* mice may represent a suitable model for the screening of the preclinical efficacy of novel drugs on the hyperactive behaviour linked to manic episode of bipolar disorder, schizophrenia and schizoaffective disorder.

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications, referred in the text by their roman numerals:

I Maksimovic, M., Vekovischeva, O.Y., Aitta-Aho, T., Korpi, E.R., 2014. Chronic treatment with mood-stabilizers attenuates abnormal hyperlocomotion of GluA1-subunit deficient Mice. PloS one 9, e100188.

II Maksimovic, M., Aitta-Aho, T., Korpi, E.R., 2014. Reversal of novelty-induced hippocampal c-Fos expression in GluA1 subunit-deficient mice by chronic treatment targeting glutamatergic transmission. Eur J Pharmacol 745, 36-45.

III Procaccini, C.*, **Maksimovic, M**.*, Aitta-Aho, T., Korpi, E.R., Linden, A.M., 2013. Reversal of novelty-induced hyperlocomotion and hippocampal c-Fos expression in GluA1 knockout male mice by the mGluR2/3 agonist LY354740. Neuroscience 250, 189-200. *equal contribution

ABBREVIATIONS

5-HT	5-hydroxytryptophan, serotonin
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANOVA	analysis of variance
BPD	bipolar disorder
CA	cornu ammonis of hippocampus
CaM kinase II	Ca^{2+}/calmodulin-dependent kinase II
CeL	central nucleus of amygdala, lateral
DA	dopamine
DG	dentate gyrus
EAAT	excitatory amino acid transporters
EPM	elevated plus maze
FST	forced swimming test
GABA	γ-aminobutyric acid
GSK-3	glycogen synthase kinase-3
IEG	immediate early gene
IP3	1,4,5-trisphosphate
LA	locomotor activity
LTP	long-term potentiation
mGluR	metabotropic glutamate receptor
NMDA	N-methyl-D-aspartate
NP	nose-poke
NSF	N-ethylmaleimide sensitive fusion protein
PFC	prefrontal cortex
PICK1	protein interacting with C kinase 1
PKC	protein kinase C
PLC	phospholipase C
PPI	pre-pulse inhibition
PSD	post-synaptic density protein
RW	running wheel
SAD	schizoaffective disorder
SCZ	schizophrenia
Ser	serine
TARP	transmembrane AMPA receptor regulatory protein
TST	tail suspension test
V	visit
VTA	ventral tegmental area
WT	wild-type line

1. INTRODUCTION

The amino acid glutamate is the major constituent of the neurotransmitter system in our brain that mediates an excitatory signal from presynaptic to postsynaptic neurons. Glutamatergic neurotransmission is fundamentally involved in the neuroplasticity occurring in development as well as in learning and memory. In the 1980s however, the glutamatergic system was also recognised to exert a role in psychiatric conditions since the compounds blocking the N-methyl-D-aspartate (NMDA) type of glutamatergic ionotropic receptors evoked psychotic symptoms resembling those encountered in schizophrenia (SCZ). This formed the basis of the glutamatergic theory of schizophrenia and this initiated an era of investigations attempting to clarify how the glutamatergic system could function in conjunction with the previous theory of dopaminergic dysfunction. Subsequently, numerous studies of patients with SCZ, bipolar disorder (BPD) and schizoaffective disorder (SAD) identified abnormalities in glutamatergic transmission in their pathophysiology. Thereafter, the glutamatergic system has become an attractive target for pharmacological treatments, via a modulation of the availability of the glutamate in the synapse, its removal from synaptic sites or modulation of its receptors. A lack of patient response to standard treatments and recurrences of symptoms highlight the clear need for the development of novel therapeutics (Gitlin, 2006; Smith et al., 2007; Tohen et al., 2005). This is particularly true since these diseases pose a substantial economic burden to the global healthcare systems and society as a whole due to the chronic course, early onset, excess mortality, remissions and probability of the severe level of disability (Wu et al., 2005).

Animal models are invaluable tools for unravelling mechanisms of psychiatric diseases, evaluating drug targets and designing selective new drugs. Unfortunately, in neuropsychiatric research, definitive animal behavioural correlates of human conditions are unattainable. However, there are two key approaches towards creating valid animal model. First is to use a model based on risk factors (such as genetic or environmental) relevant to the pathophysiology of human disease. Second, an assessment of phenotype can be undertaken with a comprehensive set of tests that have cross-species validity. The use of these models can lead to identification of drug candidates with greater translational value. Alternatively, their use may lead to the discovery or the development of improved therapeutics.

The unclear etiology, clinical heterogeneity, cyclic nature of disease and unknown biomarkers/endophenotypes for evaluating treatment outcomes represent additional challenges for modelling of BPD, SCZ and SAD. However, as our understanding grows of the role of the glutamate in these diseases, it may be possible to generate novel glutamate-modifying therapeutics and real breakthroughs as disease-modifying agents.

2. REVIEW OF LITERATURE

2.1 NEUROTRANSMITTER GLUTAMATE: SYNTHESIS, PACKAGING AND TRANSPORT

After its release from the presynaptic neuron, glutamate triggers depolarization of postsynaptic membrane and subsequently activates the postsynaptic neuron. This is in direct opposition to the GABAergic effect that hyperpolarises post-synaptic neuron; together, these two systems mediate the majority of excitatory and inhibitory transmission.

Glutamate is formed from α-ketoglutarate, an intermediate in the metabolic pathway known as tricarboxylic acid cycle (TCA) or Krebs cycle (Albrecht et al., 2007). The effect of glutamate is terminated by its re-uptake from the synaptic cleft into astrocytes, the glial cells expressing excitatory amino acid transporters (EAAT) (Slotboom et al., 1999). There, the enzyme glutamine synthetase converts glutamate to glutamine, which lacks neurotransmitter properties and this is then exported back to neurons. Neurons in turn have the ability to deamidate glutamine via phosphate-activated glutaminase (Akiyama et al., 1990), and the resynthesized glutamate is then taken up and stored into vesicles. There may also exist one other, glutamine-independent mechanism of production of glutamate in the neurons (Hassel and Brathe, 2000). This involves direct usage of α-ketoglutarate and counter-down action of neuronal pyruvate carboxylase to replenish TCA cycle with malate, a precursor of oxaloacetate. In GABAergic neurons, there is a one-step reaction of glutamate decarboxylase, which converts glutamate into GABA (Martin and Rimvall, 1993).

Inside the neurons, the neurotransmitter glutamate is concentrated into synaptic vesicles by vesicular glutamate transporters, of which three forms are currently known (VGLUT I-III) (Fremeau et al., 2002; Kaneko and Fujiyama, 2002). Their expression on GABAergic, cholinergic and monoaminergic neurons raised some doubts about them being specific to glutamatergic neurons (Fremeau et al., 2004).

Although it is involved in mediating many fundamental processes, the effects of glutamate at times can be detrimental and therefore are tightly controlled (Mark et al., 2001). The rapid removal of glutamate from the extracellular space is crucial for the optimal function of synapse and prevention of postsynaptic overstimulation. This is achieved by a family of high-affinity EAAT transporters (Danbolt, 2001). EAAT2 is the principal transporter in the forebrain and this protein is present in both astrocytes and neurons. EAAT1 is only found in astrocytes; it is abundant in the cerebellum and present in the forebrain. EAAT3 is expressed in neurons in the whole brain (Conti et al., 1998); EAAT4 is largely expressed on dendrites of cerebellar Purkinje cells (Yamada et al., 1996), whereas EAAT5 is expressed in the retina (Bringmann et al., 2009). In the rat EAAT1–3 are termed GLAST, GLT and EAAC1, respectively.

Several sites at the glutamatergic synapse are being exploited for pharmacotherapy: modulation of glutamate transport back to astrocyte, or packaging into vesicles and release, or presynaptic release or voltage-dependent Na^+ channel modulation that affect glutamate release. Additionally, one further site of modulation is located at the receptor level.

2.2 GLUTAMATE RECEPTORS

Glutamate receptors are classified into two major categories, ionotropic (AMPA, NMDA, kainate and delta) and metabotropic (mGluR), depending on whether ion channels or second messenger system transduce the glutamate signal, respectively (Table 1, 2). Ionotropic receptors are assembled from four subunits that surround a central pore: GluA1–GluA4 for α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) (formerly GLU_{A1-A4}, GluR1-4, GluRA-D), GluK1–GluK5 for kainate (formerly GLU_{K5-K7} and $GLU_{K1-K2,}$ GluR5-7 and KA1-2, GluR5-7 and KA-1-2), GluN1, GluN2A-2D and GluN3A-3B for NMDA receptors (formerly GLU_{N1}, GLU_{N2A-2D} and GLU_{N3A-3B}, NMDA-R1, NMDA-R2A-2D and NMDA-R3A-3B, and NR1, NR2A-2D and NMDAR-L) and GluD1-D2 subunits for the recently characterized delta receptors (see Collingridge et al., 2009 for detailed nomenclature of ionotropic receptors). Depending on the subunit composition, ionotropic receptors can conduct fluxes of Na^+ and Ca^{2+}. Each subunit consists of four different domains: amino-terminal, ligand-binding, transmembrane and carboxyl-terminal domain. Metabotropic receptors (mGluR1-8) are G protein-coupled receptors, and their effects can be either inhibitory or stimulatory, depending on the G protein that they recruit.

Table 1. Ionotropic glutamate receptors.

Receptor	Subunit composition	Action	Transduction mechanism	Localization	Pharmacology (Examples)
NMDA	GluN1 GluN2A GluN2B GluN2C GluN2D GluN3A GluN3B	Excitatory	Na^+ Ca^{2+}	Postsynaptic Extrasynaptic	*Agonists*: glycine, D-Serine, alanine, glutamate, aspartate *Antagonists:* - Competitive: AP5, CPP - Noncompetitive: ifenprodil - Uncompetitive: ketamine, phencyclidine *Positive allosteric modulators*: neurosteroids
AMPA	GluA1 GluA2 GluA3 GluA4	Excitatory	Na^+ (Ca^{2+})	Postsynaptic Extrasynaptic	*Agonists*: glutamate, AMPA, kainate *Antagonists:* - Competitive: CNQX, NBQX - Noncompetitive: CP-465,022, GYKI 53655, perampanel - Uncompetitive: argio-, philantho- toxins *Positive allosteric modulators:* aniracetam, ampakines, cyclothiazide
Kainate	GluK1 GluK2 GluK3 GluK4 GluK5	Excitatory	Na^+ (Ca^{2+}) K^+	Presynaptic Postsynaptic	*Agonists*: glutamate, AMPA, kainate *Antagonists:* - Competitive: CNQX, NBQX - Noncompetitive: GYKI 52466 - Uncompetitive: joro spider -toxin
Delta	GluD1 GluD2	Excitatory	Na^+ (Ca^{2+})	Inner hair cells of the organ of Corti; Highly expressed in forebrain Enriched in cerebellum	*Antagonists:* pentamidine

2.2.1 AMPA RECEPTORS

The activation of AMPA receptor produces excitatory postsynaptic potentials (EPSPs). The activation of AMPA receptors is fast and transient, causing very brief depolarisations that last no longer than a few milliseconds in contrast to a

slower rise time and decay of the NMDA component. Unlike their counterparts NMDA, AMPA receptors are crucial for basal synaptic transmission, and structural and functional modifications at AMPA receptors are actively involved in several stages of synaptic strengthening and long-term potentiation and depression that are the correlates of memory formation. See section 2.8 for more details on the properties of the AMPA receptors.

2.2.2 NMDA RECEPTORS

Most of the functional NMDA receptors are heteromeric, requiring two GluN1 subunits together with either two GluN2 or a combination of GluN2 and GluN3 subunits (Monyer et al., 1992; Schorge and Colquhoun, 2003; Ulbrich and Isacoff, 2007). Expression of GluN1 is almost ubiquitous in central nervous system, whereas the distribution of the other subunits displays a more distinctive pattern of expression. GluN2A and GluN2B are present in forebrain, GluN2A and GluN2C in cerebellum and GluN2D is present in midbrain and hindbrain. GluN3A is mainly found in spinal cord and cortex whereas GluN3B can be detected in motor neurons in the spinal cord, pons and medulla. There are numerous regulatory sites present on the NMDA receptors, e.g. recognition sites for two different agonists (glycine and glutamate) and polyamine regulatory site both of which trigger ion flux through receptor. There are also separate sites for Mg^{2+}, Zn^{2+} and H^{+} that inhibit receptor activation (Dingledine et al., 1999; Mayer, 2005). The NMDA receptors possess several unique properties; their affinity for glutamate is high and it displays very slow kinetics of (de)activation. The activation of the receptor exhibits an unusual co-agonistic action: simultaneous binding of glycine to GluN1 and glutamate to GluN2 is required (Johnson and Ascher, 1987). Another brake on its activation is provided by extracellular Mg^{2+}, which exerts a voltage-dependent block on the open ion channel (Nowak et al., 1984). The H^{+} ion is a potent inhibitor of NMDA receptors activation which may be especially important in pathological conditions such as acute stroke and other acidosis conditions. The long time-course of NMDA receptors activation provides an opportunity for temporal and spatial summation of multiple inputs. These specificities permit the NMDA receptor to act as a perfect coincidence detector: large depolarization and synaptic release of glutamate are both required to allow permeation of calcium (Ca^{2+}) ions through the ion pore (Seeburg et al., 1995). The high Ca^{2+} permeability makes possible the interaction of Ca^{2+} ions with calcium-dependent enzymes, second messengers, protein kinases and phosphatases, scaffolding proteins, cytoskeletal elements, GTP binding proteins and their regulators, and adhesion molecules which then transform the synaptic inputs into long-lasting cellular modifications.

In addition to glycine, the D- and L-isomers of serine and alanine can act as agonists at the GluN1 subunit. The agonists at GluN2 are typically short-chain dicarboxylic amino acids, such as glutamate, aspartate and NMDA itself although it is several-fold less potent than glutamate (Table 1). If one extends the length of the glutamate carbon chain, then one can obtain several competitive antagonists

at the glutamate recognition site (Traynelis et al., 2010). Numerous competitive antagonists are available (Table 1), such as D-2-amino-5-phosphonopentanoic acid, which traditionally has been used for distinguishing the NMDA from the responses mediated via AMPA and kainate receptors. The selectivity of the GluNR2 subunit is difficult to achieve but it could be one way to develop compounds with distinct therapeutic potential and side effects. The first subunit-selective NMDA receptor antagonist, ifenprodil, introduced a new class of noncompetitive antagonists of GluN2B-containing NMDA receptors; this compound binds at a site distinct from the glutamate- and glycine-binding sites (Hess et al., 1998). The compounds that block NMDA receptor channels via use-dependent and voltage-dependent mechanism such as the dissociative anaesthetics, phencyclidine (PCP) and ketamine are uncompetitive antagonists (Table 1). They slowly dissociate from their binding site in the NMDA receptor channel and may become trapped in the pore ('trapping blockers') which are features that complicate their safety profile.

2.2.3 KAINATE RECEPTORS

GluK1–GluK3 subunits form homomers or heteromers, or they co-assemble with GluK4 or GluK5, which are otherwise inactive on their own (Lerma, 2003; Traynelis et al., 2010). As with the AMPA receptors, mRNA editing at the Q/R site reduces divalent cation permeability and channel conductance. In the mossy fiber-CA3 pyramidal cell synapse, the most extensively studied site of kainate receptor-mediated synaptic transmission, GluK2 and GluK5 are located postsynaptically, whereas GluK1 to GluK3 are present presynaptically. In that way, they are able to mediate a wide range of functions, e.g. postsynaptic depolarization at a subset of excitatory synapses, presynaptic modulation of both excitatory and inhibitory transmission and refinement of synaptic strength during development (Contractor et al., 2011). The clarification of their role was delayed due to the lack of antagonists selective for kainate over AMPA receptors but recently some agents have been developed (Table 1)(Sakai et al., 2001; Zhou et al., 1997). Given the role of kainate receptors in mediating epileptiform activity, it would be interesting to identify agents that could selectively target the kainate receptor subunits in this pathophysiological condition (Vincent and Mulle, 2009).

2.2.4 DELTA RECEPTORS

This orphan family is characterised by its sequence homology with the ionotropic family subunits, however, they are unresponsive to glutamate which makes it difficult to determine their functional roles (Lomeli et al., 1993; Yamazaki et al., 1992). They consist of the GluD1 and GluD2 receptors that share 60% homology

(Table 1). GluD2 is selectively expressed in the parallel fiber-Purkinje cell synapse and it associates with the mGluR1 in the cerebellum (Takayama et al., 1996); a loss of GluD2 in this region has been linked with impairment in motor learning and severe ataxia. The deletion of GluD1 causes a hearing deficit in mice as GluD1 is highly enriched in the inner hair cells of the organ of Corti (Gao et al., 2007; Safieddine and Wenthold, 1997).

2.2.5 METABOTROPIC RECEPTORS

Three functional classes of mGluRs exist based on amino-acid sequence homology, agonist pharmacology and the signal transduction pathways to which they are coupled (Table 2). Group I includes mGluR1 and mGluR5, group II includes mGluR2 and mGluR3, and group III includes mGluR4, mGluR6, mGluR7 and mGluR8. It is known that alternatively spliced variants exist for mGlu1, mGlu4, mGlu5 and mGlu7 (Kew and Kemp, 2005). The group I mGluRs couple with G_q protein, stimulate phospholipase C (PLC) activity to produce 1,4,5-trisphosphate (IP3), further releasing the Ca^{2+} from cytoplasmic stores, as well as diacylglycerol, which activates the enzyme PKC. The activation of the group II and III mGluRs results in inhibition of adenylate cyclase through $G_{i/o}$ protein.

The mGluRs are localised in both neurons and glia and they are present in both pre- (group II and III) and postsynaptic sites (group I) (Lujan et al., 1996; Pinheiro and Mulle, 2008). Postsynaptic mGluR activation triggers an excitatory response, whereas presynaptic mGluR activation blocks synaptic transmission. For example, the inhibition of voltage-dependent Ca^{2+} channels, the activation of potassium (K^+) and/or the inhibition of the exocytotic processes downstream from calcium entry (Conn and Pin, 1997) would exert negative feedback to ensure that the glutamate transmission remains within the physiological range.

Table 2: Metabotropic glutamate receptors (Modified from Swanson et al., 2005).

Subtype	Structure	Action	Transduction mechanism	Localization	Pharmacology (Examples)
GROUP I					
mGluR1 mGluR5	G protein-coupled receptors (seven transmembrane domain)	Increase excitability (Gq-coupled)	Activation of PLC (increase Ca^{2+}, IP3)	Postsynaptic at glutamatergic synapses; mGluR5 also in glial cells mGluR1: Required for LTD in cerebellar synapses mGlu5: Highly expressed in forebrain	*Agonists*: DHPG, 1S,3R-ACPD, CHPG (mGluR5) *Antagonists*: LY393675 *Inverse agonist (or allosteric antagonist):* LY367385 (mGluR1), MPEP (mGluR5)
GROUP II					
mGluR2 mGluR3	G protein-coupled receptors (seven transmembrane domain)	Decrease excitability (Gi/o-coupled)	Inhibition of adenylate cyclase; Inhibition of voltage-gated-Ca^{2+}channels; Activation of K^+ channels	Presynaptic regulation of glutamate and GABA release, also postsynaptic mGluR2: Highly expressed in forebrain mGluR3: Widely expressed in glial cells	*Agonists*: DHPG, 1S,3R-ACPD, LY354740 *Antagonists*: LY341495
GROUP III					
mGluR4 mGluR6 mGluR7 mGluR8	G protein-coupled receptors (seven transmembrane domain)	Decrease excitability (Gi/o-coupled)	Inhibition of adenylate cyclase; Inhibition of voltage-gated-Ca^{2+}channels; Activation of K+ channels	Pre- (mGluR4, 7, 8) and post-synaptic (mGluR4, 7) on glutamatergic and other systems mGluR6: Only in the retina mGluR4:Highly expressed in cerebellum mGluR7: Has lower affinity for glutamate than other subtypes mGluR8: Highly expressed in forebrain	*Agonists*: L-AP4, ACPT-1 (mGluR4), 3,4-DGPG (mGluR8) *Antagonists*: MSOP, CPPG (mGluR4), LY341495 (mGluR7; 100-fold lower affinity than group II)

2.3 AMPA RECEPTOR PROPERTIES

2.3.1 LOCALISATION OF AMPA RECEPTORS

The mRNAs encoding GluA1-A4 protein subunits of AMPA receptors are abundantly present in the central nervous system, as detected by *in situ* hybridisation and immunohistochemical methods; hippocampal formation, the cortical regions, the basal ganglia, the olfactory bulbs and the amygdala are particularly enriched with these receptors (Keinanen et al., 1990; Petralia and Wenthold, 1992). The principal AMPA channels at excitatory neurons are heteromers comprised of the GluA2 subunit and one of the GluA1, GluA3 or GluA4 subunits. The homomers of GluA1 are located on inhibitory neurons whereas those of GluA3 and GluA4 receptors are present at only low levels (Fleming and England, 2010; Keinanen et al., 1990). The expression of the GluA2 subunit determines Ca^{2+} permeability and its levels increase throughout the early postnatal period.

AMPA receptors are found at synaptic, extrasynaptic and intracellular sites. Synaptic receptors are predominantly GluA1A2-containing (about 80%) (Beique and Huganir, 2009; Lu et al., 2009) with the remaining receptors being GluA2A3-containing. There are extrasynaptic receptors, which are overwhelmingly GluA1A2 heteromers, and in conjunction with the intracellular receptors, they form a reserve pool for trafficking of AMPA receptor into and back out of synapses (see chapter 2.3.5 below for more details on this topic). The composition of AMPA receptor subtypes is an important aspect of synaptic plasticity.

2.3.2 SYNTHESIS OF AMPA RECEPTORS

Individual subunits are synthesized in the cell body and the assembly of the channel continues in the endoplasmic reticulum. Palmitoylation, a posttranslational modification, is important for ensuring correct trafficking of the AMPA receptor (Hayashi et al., 2005) and this process occurs in the Golgi. However, subunits can also be synthesized locally in dendrites from mRNA that has been transported from the cell body. Indeed, all of the required translational machinery and Golgi are present in dendrites. The local synthesis of AMPA receptor subunits apparently maintains local receptor abundance and composition (Ju et al., 2004).

2.3.3 STRUCTURE OF AMPA RECEPTORS

The amino-terminal domain is made up of a large number of amino acids of each subunit of the AMPA receptor, but has a more regulatory role and it is not essential for receptor function (O'Brien et al., 1999) (Fig. 1). Receptor heterogeneity is achieved in this domain by alternative splicing and mRNA editing (Traynelis et al., 2010). The flip and flop splice variants differ in 11 amino acids in the extracellular loop between the transmembrane M3 and M4 segments and these changes influence the rate of receptor desensitisation and regional distribution. The expression of these variants is dependent on the developmental stage i.e. the flip form is present in prenatal AMPA receptors whereas the numbers of the flop variant increase postnatally (Monyer et al., 1991). RNA editing, located near the flip/flop splicing site, results in a change from arginine to glycine (R/G) in the GluA2-GluA4 subunits (Lomeli et al., 1994), which modifies the desensitisation properties of the receptors. The edited G forms recover more quickly from desensitisation. The ligand-binding domain lies in clamshell-like gorge formed by two lobes, S1 and S2. Agonist binding causes a partial closure of the clamshell and opens the pore. The channel-lining M2 domain, which harbours the Q/R site, has an unusual re-entrant loop with both ends facing the cytoplasm. The 'Q/R site' determines Ca^{2+} permeability. If glutamine (Q) resides in this position as is the case in GluA1, GluA3 and GluA4, the channel is permeable to Ca^{2+}, but if arginine (R) is present as in GluA2, it does not permit the Ca^{2+} to pass through the channel. Since the GluA2 subunit is predominantly found in most of the AMPA receptors, they are impermeable to Ca^{2+}. In fact, increased AMPA fluxes due to the appearance of Ca^{2+} permeable, GluA2-lacking receptors are found not only in some pathological conditions such as cerebral ischaemia (Gorter et al., 1997) and epilepsy (Friedman et al., 1994; Prince et al., 1995) but also after drug-induced potentiation in the glutamatergic synapses on ventral tegmental area (VTA) dopamine (DA) neurons (Vashchinkina et al., 2012). The carboxyl-terminal domain is important for binding with the proteins that influence receptor modification and trafficking. There is also another alternative splicing called the "short" and "long" splice variants in GluA1, GluA2 and GluA4 subunits (Kohler et al., 1994). An additional mechanism for tuning of the receptor is achieved via a number of transmembrane proteins. Transmembrane AMPA receptor regulatory proteins (TARPs) and cornichon homologs regulate gating, ion permeability, pharmacology and trafficking of the receptor (Traynelis et al., 2010).

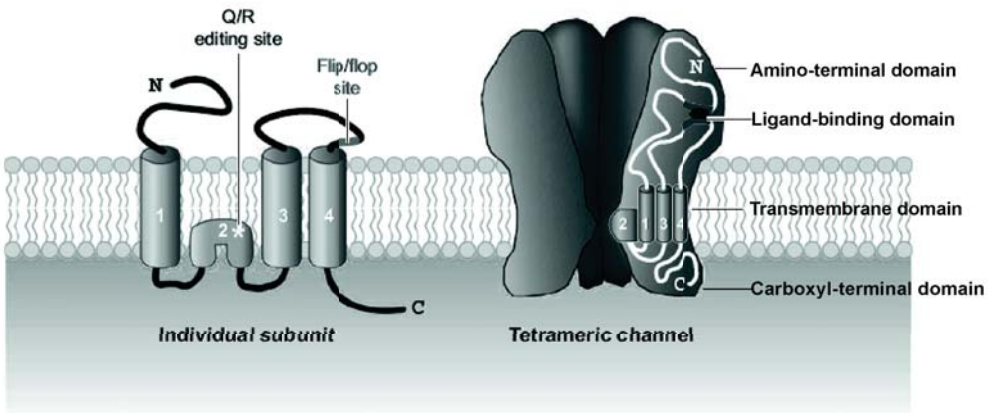

Figure 1. Structure of the AMPA receptor subunits and the tetrameric channel. The channel consists of four subunits, which are usually two dimers of two different subunits, such as GluA1 and -2 or GluA2 and -3. The individual subunits are composed of four transmembrane domains. The N-terminal domain lacks a clear functional role. Two alternatively spliced versions, flip and flop, influence the pharmacological and kinetic properties of the channel and form part of the extracellular ligand-binding domain. Editing of a glutamine codon to an arginine codon (Q/R editing site) in the ion channel pore region regulates the calcium permeability. The AMPA receptor C-termini differ in their amino acid sequence, and this determines the nature of their interacting partners. Modified from Shepherd and Huganir, 2007.

2.3.4 PHARMACOLOGY OF AMPA RECEPTORS

Quinoxalinediones are widely used competitive AMPA receptor antagonists (CNQX, DNQX, NBQX), which are selective in the sense that they do not bind to NMDA receptors but they do antagonise kainate receptors; NBQX seems to have greater selectivity for AMPA receptors (Wilding and Huettner, 1996) (Table 1). Noncompetitive antagonists at AMPA receptors have been used to selectively target AMPA but not kainate receptors, including 2,3-benzodiazepines and 1,2-dihydrophthalazines, as well as tetrahydroisoquinolines (Gitto et al., 2003), with CP-465,022 apparently showing greater selectivity than GYKI-53655 for AMPA in preference to kainate receptors (Lazzaro et al., 2002). The subsequent generation of noncompetitive AMPA antagonist, perampanel and talampanel, have been reported to be highly selective for AMPA receptors (Rogawski and Hanada, 2013; Swanson, 2009) (Table 1). Perampanel has been recently approved for the treatment of partial onset seizures. Since the ligand-binding domain sequence is highly conserved among all four GluA subunits, currently there is no agonist possessing subunit selectivity, even though some of these compounds were developed to discriminate between GluA1/GluA2 and GluA3/GluA4 subunits (Coquelle et al., 2000). Furthermore, the association of AMPA receptors with TARPs affects agonistic affinity (Kott et al., 2007; Turetsky et al., 2005), a property which was underestimated in *in vitro* studies. Positive allosteric modulators

(aniracetam, piracetam, ampakines, cyclothiazide) were developed in attempts to prevent the desensitisation state i.e. the phenomenon when agonist remains bound but the channel is closed.

2.3.5 AMPA RECEPTOR TRAFFICKING

AMPA receptors are continuously being trafficked (endocytosed, recycled, and reinserted) into and out of the plasma membrane in a regulated and a constitutive manner (Malenka, 2003). In the regulated pathway, GluA1-containing AMPA receptors are trafficked to the synapse in an activity-dependent manner, which is stimulated by NMDA receptor activation. In contrast, GluA1-lacking AMPA receptors, usually GluA2A3 heteromers are being trafficked constitutively. These two pathways are governed by the interactions between the carboxyl-terminal domain of subunit and various synaptic compounds and proteins. The short C-tails of GluA2/3 receptors allow them to be inserted directly into the post-synaptic density protein (PSD) in an interaction with N-ethylmaleimide sensitive fusion protein (NSF), which dissociates GluA2 from protein interacting with C kinase 1 (PICK1). The presence of long C-tails prevent GluA1/4 receptors from being inserted directly into the synapse in the absence of activity and in fact they are first inserted at extrasynaptic adjacent sites and laterally diffuse to synapses (Borgdorff and Choquet, 2002; Passafaro et al., 2001). The constitutive pathway maintains total number of synaptic AMPA receptors, and the regulated pathway is evoked transiently upon the induction of synaptic plasticity.

2.3.6 THE POSTSYNAPTIC DENSITY

NMDA and AMPA receptors are located extensively across the postsynaptic density (PSD), whereas mGluRs (except mGluR7) are concentrated along the periphery of the PSD. PSD is a protein complex containing machinery for transduction of the signal from receptors into various intracellular signal pathways (Fig. 2). Thus, the PSD can be considered to consist of ion channels, glycolytic enzymes, transporters, proteins of intracellular signaling pathways, cell adhesion molecules and the scaffolding proteins that link together the proteins of the PSD (Kennedy, 2000; Walikonis et al., 2000).

One major scaffolding protein of the PSD is PSD95 that is anchored to the lipid rafts via palmitic acid residues (Fig. 2). This protein has several domains through which it can bind to other proteins: PDZ domain (short for PSD95/disc large/zona occludens-1) that binds C-termini of other proteins, the Src homology domain, and the guanylate kinase domain. PSD95 anchors the NMDA receptor via PDZ domain to the cytoskeleton of the dendritic spine and couples it to the signaling effector cascade. The Ca^{2+} that has entered into the postsynaptic cell after NMDA receptor

activation interacts with protein calmodulin and activates a protein kinase, Ca^{2+}/calmodulin-dependent kinase II (CaM kinase II). It is bound to α-actinin via the scaffolding protein densin. The target of this PSD abundant kinase is the serine (Ser) residue 831 of the GluA1 subunit of the AMPA receptor. This phosphorylation increases the conductance of the AMPA receptor, which is one mechanism that contributes to the potentiation of glutamatergic synapses (Palmer et al., 2005). Another important phosphorylative modification of GluA1 subunit is catalysed by PKA and PKC that are anchored by scaffolding protein A-kinase together with protein phosphatase calcineurin to the Src homology domain and the guanylate kinase domains of PSD95 (Fig. 2). Phosphorylation of GluA1 on Ser845 by PKA recruits to a variable degree more of AMPA receptors, which are located intracellularly in recycling endosomes, to the plasma membrane of the dendritic spine. PKC phosphorylates other serine residues on GluA1 (Ser816 and Ser818) enabling GluA1 to travel along actin filaments to the plasma membrane. On the other hand, Ca^{2+}-dependent activation of the phosphatase calcineurin, which dephosphorylates GluA1 on Ser845, would lead to relocation of AMPA receptors from the plasma membrane to the cytosol and long-term depression (Collingridge et al., 2010). The entry of Ca^{2+} may also activate nitric oxide synthase and adenylate cyclase (Fig. 2).

Stargazin and other members of TARP family provide an indirect link between AMPA receptors and PSD95. Other PSD proteins with PDZ domains can directly interact with the C termini of AMPA receptors. The GluA2/3 subunits interact with the glutamate receptor-interacting protein and AMPA receptor-binding protein (Dong et al., 1997; Srivastava et al., 1998). The GluA2, -3, and -4 bind with PICK1 (Xia et al., 1999), while GluA1 interacts with synapse-associated protein of 97 kDa (Leonard et al., 1998).

Scaffolding proteins Homer and Shank anchor metabotropic glutamate receptors to the periphery of the PSD (Tu et al., 1999). In addition, Homer is also the link connecting mGluRs to the IP3 receptor (Fig. 2). In this way, IP3 released upon stimulation of type I mGluRs reaches the IP3 receptor.

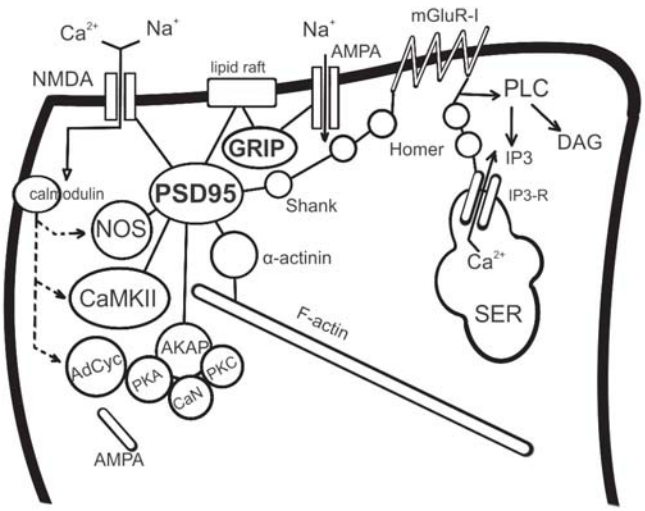

Figure 2. Schematic representation of a dendritic spine of a glutamatergic synapse with some proteins of the postsynaptic density. Lipid rafts, scaffolding proteins (such as PSD95, GRIP, Shank and Homer) and cytoskeletal proteins (F-actin) help to concentrate and stabilize effector proteins at the spine. Adenylate cyclase *(*AdCyc*)*, A-kinase anchoring protein (AKAP), AMPA receptor, Ca^{2+}/calmodulin-dependent kinase II (CaMK II)*,* calcineurin (CaN), diacylglycerol (DAG), filamentous actin *(*F-actin), glutamate receptor-interacting protein (GRIP), inositol-1,4,5-triphosphate (IP3), metabotropic glutamate receptor (mGluR), NMDA receptor, nitric oxide synthase (NOS), protein kinase A and C respectively (PKA and PKC), phospholipase C (PLC), postsynaptic density protein with a molecular weight of 95 kDa (PSD95); smooth endoplasmic reticulum (SER). Modified from Brady et al., 2012.

2.4 GLUTAMATE IN NEUROPSYCHIATRIC DISORDERS

Since the glutamate is the major excitatory transmitter, its proper functioning is fundamental for the brain i.e. in growth, development and cognition. In the last decades, there are several lines of evidence pointing to the altered glutamatergic transmission in mood and psychotic disorders, however. Brain glutamate levels are higher in bipolar manic patients and in those with SCZ than in healthy subjects as measured by *in vivo* by proton magnetic resonance spectroscopy (Gigante et al., 2012; Poels et al., 2014); the higher glutamate levels are independent of the current state, with a tendency towards reduction in response to medication. The analysis of the post-mortem frontal cortex of these patients revealed markers of excitotoxicity and neuroinflammation (Rao et al., 2010). Furthermore, genetic studies pointed to polymorphisms of GluN1 and GluN2 subunits of NMDA receptors with regard to mood disorders and SCZ and reduced expression of NMDA receptor subunits has been demonstrated (Kristiansen et al., 2007). Although there are several subunits of AMPA-type glutamate receptors, decreased expression of the GluA1 subunit has been observed in the post-mortem

hippocampus, thalamus and frontal cortex of schizophrenic patients (Dracheva et al., 2005; Eastwood et al., 1997; Eastwood et al., 1995; Harrison et al., 1991; Ibrahim et al., 2000; Sokolov, 1998) and in the striatum of bipolar patients (Meador-Woodruff et al., 2001).

The pharmacological blockade of NMDA receptors with PCP or ketamine serves as a commonly used rodent model of psychosis. Mechanistically, hypo-function of NMDA receptors would presumably decrease inhibitory tone and favour cortical over-excitation (resulting in stimulation of non-NMDA glutamate receptors) that could induce psychotic-like symptoms (Moghaddam et al., 1997). The symptoms are reversible by administration of agonists of group II mGluRs that act on presynaptic inhibitory autoreceptors and reduce excess glutamate release (Krystal et al., 1999; Moghaddam and Adams, 1998), or with an antagonist of AMPA receptors (Moghaddam et al., 1997). Even though there was convincing preclinical evidence, one clinical study of mGluR2/3 receptor agonist in SCZ was discontinued (Kinon et al., 2011; Stauffer et al., 2013), since no benefits were seen with the tested compound over standard antipsychotic therapy.

Indeed, many drugs that are used as mood-stabilisers and anticonvulsants are glutamate-modifying agents (Table 3). They have recognized effects on presynaptic release of glutamate, act on synaptic cleft, change the binding of glutamate to its receptors or regulate its transport. By blocking the use-dependent sodium channels or reducing vesicular glutamate transporter expression they stabilise membranes and control release of glutamate (lamotrigine, valproate, topiramate). These drugs favour glutamate uptake via excitatory amino acid transporters (EAAT) EAAT1 and EAAT2 to neighbouring astrocytes (valproate). Blockade of glutamate kainate/AMPA receptors (perampanel, topiramate) and the alteration of subunits expression, phosphorylation and trafficking will ultimately affect receptor function (lithium, valproate, lamotrigine). In addition to their well-established effects on GABA function (topiramate and valproate) (Macdonald and Kelly, 1995; Ueda and Willmore, 2000), it seems that the rebalancing between excitatory and inhibitory transmission is involved in mediating the therapeutic benefits of these drugs.

Table 3. The targets of selected drugs with respect to glutamatergic transmission (modified from Sanacora et al., 2008).

Drug	Effect	References
Lithium	Increases VGLUT1 expression and presynaptic glutamate release	Moutsimilli et al., 2005; Dixon et al., 1994; Hokin et al., 1996
	Acutely inhibits, chronically upregulates glutamate uptake	Dixon and Hokin, 1998
	Reduces the level of GluN2A and GluN2B subunit phosphorylation; Decreases Src and Fyn interactions with GluN2A subunit mediated by PSD-95	Hashimoto et al., 2002; Basselin et al., 2006; Ma and Zhang, 2003
	Reduces the level of GluA1 and GluA2 subunits in synapses and attenuates phosphorylation of GluA1 at its PKA site S845; reduces AMPA/NMDA ratio	Du et al., 2008; Du et al., 2003; Du et al., 2004
Valproate	Reduces VGLUT1 and glutamate release	Cunningham et al., 2003; Kang et al., 2005
	Increases levels of glutamate transporters EAAT and astrocytic uptake	Hassel et al., 2001; Ueda and Willmore, 2000
	Inhibits AMPA binding	Kunig et al., 1998
	Reduces the level of GluA1 and GluA2 subunits in synapses and attenuates phosphorylation of GluA1 at its PKA site S845;	Du et al., 2008; Du et al., 2003; Du et al., 2004; Du et al., 2007
	Blocks NMDA receptors	Ko et al., 1997; Steppuhn and Turski, 1993; Zeise et al., 1991
Lamotrigine	Blocks voltage-gated sodium channels	Leach et al., 1991; Zona and Avoli, 1997
	Reduces glutamate release	Ahmad et al., 2005
	Inhibits AMPA receptor	Lee et al., 2008
	Increases the level of GluA1 and GluA2 subunits in synapses and enhance phosphorylation of GluA1 at its PKA site S845;	Du et al., 2007
Topiramate	Blocks voltage-gated sodium channels	Gibbs et al., 2000; Wu et al., 1998; Zona et al., 1997
	Blocks glutamate kainate/AMPA receptors	Angehagen et al., 2003; Shank et al., 2000
Perampanel	Blocks AMPA receptors	Rogawski and Hanada, 2013

Abbreviations: vesicular glutamate transporter 1 (VGLUT1), Src and Fyn (two members of the Src family of protein tyrosine kinases), post-synaptic density protein (PSD-95), PKA (protein kinase A), EAAT, excitatory amino-acid transporter.

2.5 BIPOLAR DISORDER, SCHIZOPHRENIA AND SCHIZOAFFECTIVE DISORDER

Bipolar disorder is characterised by cycles of mood swings, from episodes of mania (bipolar type I) or hypomania (bipolar type II) to depression (Table 4). The manic episode consists of long periods of feeling 'high' and excessively elevated, expansive or easily irritable mood (Diagnostic and statistical manual of mental

disorders 5th ed.; *DSM-V;* American Psychiatric Association, 2013). Other behavioural characteristics include inflated self-esteem or grandiosity (an unrealistic sense of one's capabilities), significantly decreased need for sleep, loquacity or pressured speech, excessive involvement in multiple projects and activities and highly risky pursuit of pleasurable activities (for example, activities involving drugs, alcohol, or sex). The manic episode of BPD can also include psychotic and cognitive symptoms (racing, disconnected thoughts, the flight of ideas, distractibility). SCZ is a life-long and debilitating psychotic illness, which exhibits both positive symptoms, such as hallucinations, delusions and racing thoughts, as well as negative symptoms (affective flattening, avolition, social withdrawal) and cognitive deficits (Table 4). A third category - SAD was first introduced in *DSM–III* (1980) (3rd ed.), including the cases with mood episodes in addition to psychotic symptoms. In the next manual, *DSM–III–R* (1987) (3rd ed., revised), four diagnostic criteria and two subtypes (bipolar and depressive) of SAD were introduced. In the *DSM-IV* (1994) (4th ed.) and *DSM-IV-TR* (2000) (4th ed., text rev.) mixed episodes within the bipolar type category were added. From that time, SAD has remained as a controversial category with constant efforts being made to improve the diagnostic criteria in order to differentiate it from SCZ and mood disorders and thereby to increase diagnostic reliability (Malaspina et al., 2013). In an attempt to resolve this discrepancy, in the newest edition (*DSM-V)* the more stringent criteria were defined with regard to the duration of the mood episode in the total course of the illness (Table 4).

Nonetheless it is still often difficult to distinguishing SAD from SCZ and from mood disorder with psychotic symptoms. In addition, affective symptoms can be identified within SCZ and psychotic symptoms can occur in conjunction with mood disorders. Thus, both psychosis and mood disturbance may constitute core symptoms of BPD, SCZ, and SAD and, in fact, many clinicians support the concept that there is a continuum with little or no rigid boundaries between these entities (Bellivier et al., 2013; Lake and Hurwitz, 2007; Malhi et al., 2008). This is evidenced by overlapping pathophysiological mechanisms of psychotic and affective disorders emerging from neurochemical, neuroimaging, neurodevelopmental, psychopharmacological, epidemiological and genetic studies (Altshuler et al., 1998; Arnone et al., 2009; Baumann and Bogerts, 1999; Berrettini, 2004; Blumberg et al., 2003; Brambilla et al., 2001; Craddock et al., 2006; Fornito et al., 2009; Strakowski et al., 1999; Walker et al., 2002; Wright et al., 2000). For this reason, the term *schizoaffective* will be used throughout this manuscript, as an umbrella term to describe both schizophrenia-like symptoms and affective symptoms. Furthermore, the term *psychotic disorders* will be used to emphasize the key phenotypic similarities.

Major endeavours have been undertaken to identify affected genes, being largely conducted by microarray analysis in post-mortem brains of patients. The confounding effects of medications and the inability to detect markers during a specific state (e.g. during a manic episode) complicate interpretation of the results, but nevertheless, these studies have revealed several genes apparently implicated in pathology of SCZ and BPD (Lin et al., 2012). The majority of genes code for

Table 4. Highlights of diagnostic criteria for bipolar mania, schizophrenia and schizoaffective disorder according to *DSM-V.*

Bipolar disorder	Schizophrenia	Schizoaffective disorder
Manic (Bipolar I)/*hypomanic* (Bipolar II) episode: A. Abnormally and persistently elevated and irritable mood lasting for at least 1 week (mania) or 4 days (hypomania) B. Three (or more) of the following symptoms: - increased goal-directed activity or energy - elevated self-esteem or grandiosity - be more talkative than usual - have flight of ideas - a reduced need for sleep - be easily distracted - psychomotor agitation - excessive involvement in activities with potential painful consequences C. Mood disturbance cause marked impairment in social and occupational functioning (for Bipolar I) *Depression episode:* A. Five (or more) symptoms for at least 2 weeks: - depressed mood - diminished interest - insomnia or hypersomnia - fatigue or loss of energy - feeling of worthlessness or guilt - diminished ability to think or concentrate B. Marked impairment in social and occupational functioning	A. Two (or more) of the following, each present for a significant portion of time during a 1-month period: - hallucinations - delusions - disorganized speech - grossly disorganized or catatonic behavior - negative symptoms (diminished emotional expression or avolition) B. For a significant portion of the time major areas of functioning, such as work, interpersonal relations, or self-care, are markedly below the level achieved prior to the onset. C. Continuous signs of the disturbance persist for at least 6 months (this include at least 1 month of symptoms)	A. An uninterrupted period of illness with a major mood episode (major depressive or manic) concurrent with criteria A for schizophrenia. The major depressive episode must include depressed mood. B. Delusions or hallucinations for 2 or more weeks in the absence of a major mood episode during the lifetime duration of the illness Note: this criterion distinguishes SAD from bipolar disorder with psychotic features. C. Symptoms that meet criteria for a major mood episode are present for the majority of the total duration of the active and residual portions of the illness Note: this criterion distinguishes SAD from schizophrenia. *Bipolar type*: if a manic episode is present (major depressive episodes may also occur) *Depressive type*: if only major depressive episodes are part of the presentation.

proteins involved in synaptic signalling and neuronal and glial functions but also in cellular metabolic processes. The most consistently reported genes have been those related to γ-aminobutyric acid (GABA) and glutamatergic neurotransmission, e.g. the expressions of alpha 5 GABA receptor (coded by GABRA5 gene) and mGluR3 metabotropic glutamate receptor (coded by GRM3 gene) have been claimed to be increased in the prefrontal and the anterior cingulate cortex in BPD (Choudary et al., 2005) whereas their expression appears to be downregulated in the prefrontal cortex (PFC) and the entorhinal cortex in SCZ (Duncan et al., 2010; Hemby et al., 2002). Furthermore, the expression of glutamate decarboxylase-1 has been consistently reported as being downregulated in both SCZ and BPD (Duncan et al., 2010; Hashimoto et al., 2008; Vawter et al., 2002). The candidate-gene based population and family association studies have also hinted at the involvement of some ionotropic glutamate receptor genes (GRIN1, GRIN2A,

GRIN2B and GRIK3), metabotropic glutamate receptor genes (such as GRM3) and GABA genes (e.g. GAD1 and GABRB2) in both illnesses (Cherlyn et al., 2010).

Rather than individual neurotransmitter implications in etiology, current research is focusing on cellular plasticity cascades that could explain dysfunctions in the circuit and behavioural levels that lead to profound motor, cognitive and affective deficits (Schloesser et al., 2008). In fact, the standard and still most widely prescribed mood-stabiliser medications seem to exert convergent effects on signalling pathways that in turn regulate the major neurotransmitter systems and thus affecting, preferably stabilising the mood. Those signalling pathways include protein kinase C (PKC) and myristoylated alanine-rich C kinase substrate, glycogen synthase kinase-3 (GSK-3), cAMP response element-binding protein and brain-derived neurotrophic factor, extracellular receptor coupled kinase and the Bcl-2 family of proteins (Schloesser et al., 2008). However, these complex phenotypes are greatly influenced by multiple genes that together with environmental experiences define individual disease susceptibility (Ginsberg et al., 2012).

2.6 HISTORY AND CURRENT PHARMACOTHERAPY OF BIPOLAR DISORDER, SCHIZOPHRENIA AND SCHIZOAFFECTIVE DISORDER

In the late 19th century, the first prophylactic effects of lithium in the treatment of recurrent symptoms of mania and depression (Lenox and Watson, 1994) were observed. The clinical use of lithium in psychiatry was later disputed but it was rediscovered in 1949 when John Cade, the psychiatrist, used lithium to solubilise uric acid while studying its role in mania; he reported that animals became tranquilised. Lithium efficacy has subsequently been confirmed in many clinical trials and for decades it remained standard therapy in BPD. Since it is a small ion, lithium can penetrate into neurons through voltage gated sodium channels and it is thought to interact with many different intracellular compounds. Even though its precise targets are still being debated, inositol phosphatases and GSK-3 are two putative candidates (Ryves and Harwood, 2001). By inhibiting GSK-3, lithium can affect insulin and Wnt signaling and exert effects on metabolism as well as on developmental and neuronal processes (Gurvich and Klein, 2002). The depletion of the phosphoinositide precursor inositol and blockade of phospholipase-dependent signalling are induced by another mood-stabiliser, valproate, although it is believed that primarily its antiepileptic effects are due to blockade of sodium channels. Those two drugs are the first line treatment for BPD, but they are also used for treating SCZ and SA disorder. The second-generation antipsychotics including olanzapine, quetiapine, risperidone, aripiprazole and the third-generation drug, asenapine, are used as adjuncts to mood-stabilisers. Two anticonvulsant drugs, lamotrigine and topiramate are newer approved treatments being utilized mainly during the maintenance phase of the illness (Dursun and Deakin, 2001).

The treatment of SCZ changed dramatically in the mid-1950s with the introduction of the first antipsychotic agent, chlorpromazine, which was initially developed as an antihistaminic (Ban, 2007). As a result, a plethora of agents were subsequently synthesized. All of these agents antagonise the DA D2 receptor subtype in the mesolimbic dopaminergic system, which has been proven to be essential for the alleviation of positive symptoms, hallucinations and delusions (Howes and Kapur, 2009). However, their use is associated with severe side effects such as the extrapyramidal syndrome and hyperprolactinaemia due to blockade of other DA pathways, the nigrostriatal and tuberoinfundibular, respectively. The safety profile was partly improved with the appearance of the second-generation antipsychotics that simultaneously blocked both D2 DAergic and 5-HT2A serotonergic (5-HT) receptors, possibly with higher affinity for the latter receptors (Meltzer, 1996). The blockade of 5-HT2A receptors is thought to release the dopaminergic cells in the nigrostriatal DA pathway from 5-HT-induced inhibition and the resulting increase in DA release may reduce the extrapyramidal syndrome. Others have proposed that a drug with low affinity for D2 DA receptors and faster dissociation could possess better therapeutic efficacy with a lower risk of side-effects (Kapur and Seeman, 2001). These so-called “atypical antipsychotics”, along with their typical counterparts, have different binding affinities towards other receptors, including D1 receptors, α1-adrenergic receptors, H1-histaminergic receptors, muscarinic receptors and many of the 5-HT receptor subtypes, which contributes to their many other side-effects (Miyamoto et al., 2012; Pratt et al., 2012). However, atypical antypsychotics lack efficacy to combat the cognitive and negative symptoms of schizophrenia and the treatment of these symptoms still remains a largely unmet clinical need (Pratt et al., 2012). Aripiprazole, referred to as a third-generation antipsychotic agent, in that sense is specific as it is a partial agonist of the D2 receptor. Not only does it reduce the hyperactive mesolimbic dopaminergic pathway, but it also activates the hypoactive mesocortical dopaminergic pathway, which it thought to be associated with negative symptoms and cognitive impairment. Aripriprazole also acts as an antagonist of 5-HT2A receptors and a partial agonist of 5HT1A receptors. It has been associated with less weight gain and fewer metabolic side effects than the older generation drugs (Swainston Harrison and Perry, 2004).

There are few controlled clinical studies which support the use of antipsychotic drugs and mood-stabilisers for the treatment of patients with SAD, depending on the specific subtype of the SAD (Levinson et al., 1999).

2.7 STRATEGIES IN THE DEVELOPMENT OF NOVEL DRUGS

The current pharmacotherapy of psychotic disorders is reliant on the drugs first discovered decades ago. Although their targets are being re-examined and novel mechanisms revealed, the plight of patients who are treatment resistant remains unanswered. Nonetheless, the future of drug development must be based on developing comprehensive, relevant and informative animal models.

The variable response to antipsychotics, the suboptimal treatment of negative and cognitive symptoms and the possibility of serious side effects, as well as the failure of disturbed monoamine signalling hypothesis to explain phenotypic complexity have all contributed to a shift of drug targeting towards modulation of major excitatory and inhibitory transmission (Coyle and Duman, 2003). The evidence that the dopaminergic disturbances are in fact preceded by glutamatergic dysregulation was the stimulus to evaluate the therapeutic properties of a variety of compounds that modulate the function of glutamatergic synapse. The potential targets for drug development are shown in Fig. 3. Modulation of presynaptic vesicular loading of glutamate, modulation of presynaptic vesicular glutamate release, voltage-dependent Na^+ channel modulation that regulates glutamate release, modulation of extrasynaptic glutamate release, modulation of group I mGluR, post-synaptic density proteins, AMPA receptor modulation, synaptic/extrasynaptic NMDA receptor modulation, facilitation of glutamate clearance by EAATs and modulation of group II mGluR can all potentially be exploited (see Fig. 3).

Administration of glycine, the glutamate co-agonist at NMDA receptors, is one approach which can be used to boost the NMDA function on GABAergic interneurons (Noetzel et al., 2012; Tsai and Lin, 2010). In order to circumvent the poor pharmacokinetics of glycine, many drug companies have tried to develop glycine transporter inhibitors (sarcosine, bitopertin) that would elevate the concentration of glycine in synapse (Lane et al., 2005; Lane et al., 2006). Another approach which can be applied to regulate NMDA receptor function is to target group V metabotropic glutamate receptors that both physically (via binding to scaffolding proteins Homer and Shank) and functionally interact with ionotropic receptor (Ehlers, 1999). These kinds of positive allosteric modulators of mGluR5 receptors would display more specific binding and thus potentiate NMDA responses (Kinney et al., 2005). Many of these agents have progressed to clinical trials, but problems with *in vivo* safety and propensity to induce convulsions have limited exploitation of their therapeutic potential (Rook et al., 2013). Alternatively, drugs with preferential effects at alpha-2 subunit-containing GABA receptors would enhance GABAergic tone on glutamatergic projection neurons and decrease the downstream effects of glutamate receptor activation (Lewis et al., 2005). In addition, agonists at group II of mGluR, mGluR2/3 agonists, proved useful in preclinical models predictive of antipsychotic activity, by removing excessive levels of glutamate in PFC, thought to underlie some of positive schizophrenic symptoms (Schoepp and Marek, 2002; Fell et al., 2012).

Minocycline, a tetracycline-type antibiotic, unexpectedly exerted both neuroprotective and anti-inflammatory effects counteracting the activation of microglia and neuroinflammatory process involved in mood disorders (Hewlett and Corbett, 2006; Molina-Hernandez et al., 2008; Tikka and Koistinaho, 2001). Minocycline was able to block the actions of an NMDA antagonist in rats (Zhang et al., 2007) and it could reverse PCP-induced cognitive deficits (Fujita et al., 2008).

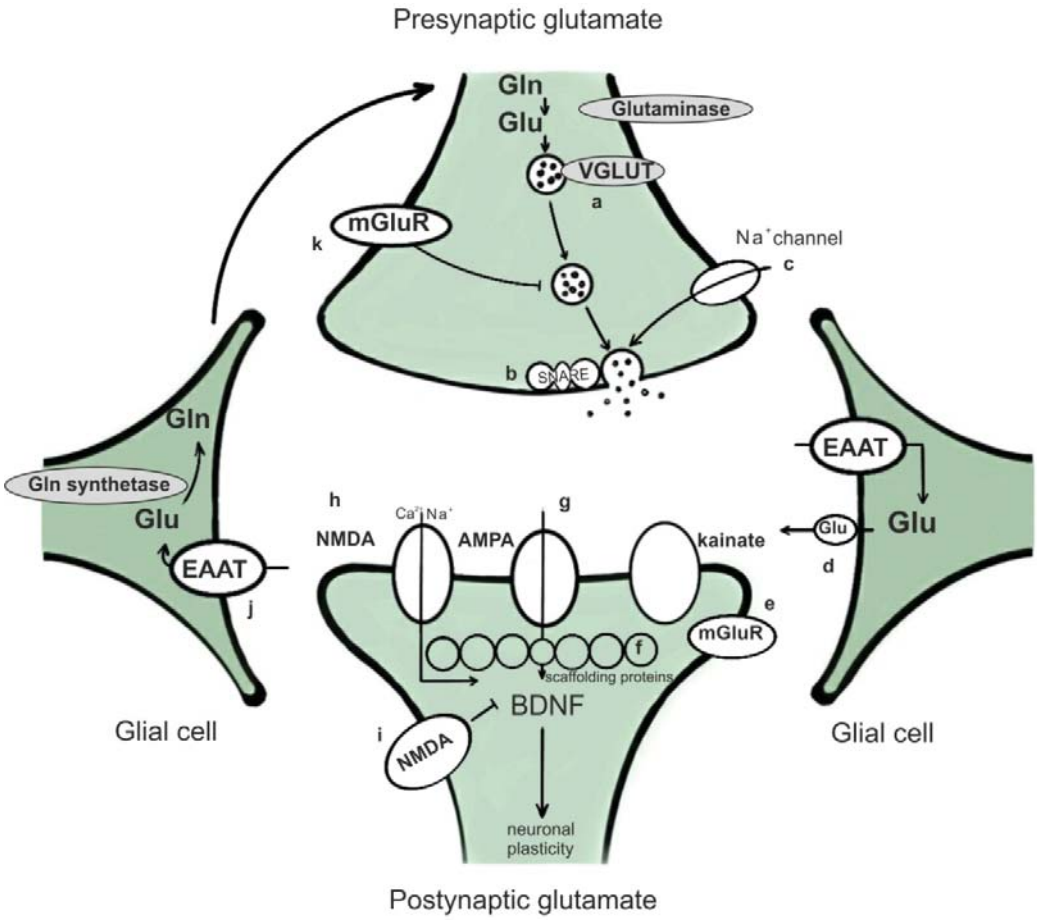

Figure 3. The glutamatergic synapse and targets for drug development: modulation of presynaptic vesicular loading of Glu (a); modulation of presynaptic vesicular Glu release (b); voltage-dependent sodium channel modulation that regulates Glu release (c); modulation of extrasynaptic Glu release (d); Group I mGluR modulation (e); post-synaptic density proteins (f); AMPA receptor modulation (g); synaptic NMDA receptor modulation (h); extrasynaptic NMDA receptor modulation (i); facilitation of Glu clearance by EAATs (j); Group II mGluR modulation (k). Gln (glutamine), Glu (glutamate), VGLUTs (vesicular Glu transporters), SNARE (N-ethylmaleimide-sensitive factor attachment receptor proteins), EAATs (excitatory amino-acid transporters). Modified from Sanacora et al., 2008.

Several other neuromodulators have emerged that target estrogen receptor or cannabinoid system (Roser et al., 2010; Zuardi et al., 1991). Oestrogen displayed some neuroprotective effects *in vitro* and *in vivo* and efficacy in neurological and neurodegenerative disease. The interest in the cannabinoids originates from the findings of increased incidence of SCZ with cannabis smoking (Moore et al., 2007). Cannabidiol may also modulate glutamatergic transmission, as it has been shown to inhibit ketamine and MK-801-induced effects in animals (Long et al., 2006; Moreira and Guimaraes, 2005).

Since there is some data indicating that there are elevated cortical glutamate levels specifically in the early phases of the illness (Theberge et al., 2003) and in the prodrome (Stone et al., 2009), it has been speculated that drugs that could reduce cortical glutamate release (such as mGluR2/3 agonists), or inhibit its effects (such as AMPA antagonists and possibly minocycline) could represent be disease-modifying agents if administered early enough in the course of disease progression (Stone, 2011).

2.8 ANIMAL MODELS OF SCHIZOAFFECTIVE PHENOTYPE

An ideal animal model of disease should fulfil three validity criteria – (1) to have symptoms (*face validity*), (2) to display a common etiology with human disease (*construct validity*) and (3) to respond to current pharmacotherapeutic agents and procedures (*predictive validity*).

Face validity means that the model has to recapitulate important anatomical, biochemical, neuropathological or behavioural features of a human disease. It is very unlikely that any animal model of a neuropsychiatric disorder would recapitulate human disease with respect to its complex symptomatology. Therefore, the validation of separate tests for separate symptoms of the disease that together form a comprehensive battery is commonly utilized (Gould and Einat, 2007).

In the past, most of models of schizoaffective phenotype focused on hyperactivity as a dominant positive symptom, and although these appeared to be useful in predicting efficacy of therapeutics (*predictive validity*), current research has taken endophenotype (intermediate phenotype) approach. This entails attempting to identify any biochemical, anatomical or endocrine markers, heritable and state independent (Gould and Gottesman, 2006), in order to increase *construct validity* linking the model to human pathophysiology. However, the etiologies of BPD, SCZ and SAD are still unclear.

In general, these animal models are mostly classified into four main categories: pharmacological, environmental, nutritional and genetic models (Kato et al., 2007). While stimulant-induced models of hyperactivity have been traditionally exploited for estimating drug efficacy, they largely lack etiological relevance. In addition, the current belief is that the effect of nutritional and environmental factors also combines with genetic factors in determining disease susceptibility. Nonetheless, the detected susceptibility genes from gene-expression profiles obtained from patient cells and tissues, despite their limitations, provide valuable data that are used in turn to produce rodent models with manipulated genes. Such reverse translation strategies are useful ways to circumvent the problem of the functionality at the brain-circuitry level (Lin et al., 2012) and these genetic models possess greater construct validity.

Antagonism of NMDA receptors with MK-801, PCP and sub-anaesthetic dose of ketamine produce locomotor hyperactivity in rodents and promote or exacerbate SCZ-like symptoms in humans. The NMDA antagonists induce the efflux of

glutamate in the prefrontal cortex, where it acts on non-NMDA receptors further eliciting dopamine release (Moghaddam and Adams, 1998). The effects can be abolished by administration of an antagonist of the AMPA/kainate receptor. Furthermore, the behavioural hyperactivity after blockade of NMDA receptors can be attenuated by lithium and valproate, and by group II mGluR agonist (Ghedim et al., 2012; Moghaddam and Adams, 1998). This model represents a well-accepted and widely used rodent model of psychosis and it reconciles, at least partly, all of the validity criteria. Moreover, it places the dopaminergic system in a secondary position to the glutamatergic system but it also confirms the close interaction between these two transmitters. Recently, several glutamatergic models have been exploited, shifting the focus of research away from dopaminergic system.

Complete animal behavioural correlates of human behaviour are unattainable, but hyperactivity, social behaviour, cognitive deficits, aggressive behaviour, risk-taking, anxiety-like and depression-like behaviours are commonly used to test for schizoaffective symptoms. Table 5 summarises genetic glutamatergic mouse models and lists the face and predictive validity for the aforementioned symptoms. Mouse models with targeted mutagenesis of glutamate receptors or proteins interacting with glutamate receptors that indirectly affect the receptor function are presented. These genetic models also make it possible to study the genes that regulate brain development and which are involved in pathogenesis of psychotic disorders. Due to the global deletion of these genes, the compensatory mechanisms might hamper the interpretation of the adult phenotype. It has been speculated that to overcome these disadvantages, time- or region-specific gene manipulation would permit better control over gene function in the examining the onset of disease or symptom manifestation (Havekes and Abel, 2009).

Table 5. Highlights of glutamatergic genetic rodent models of schizoaffective symptomatology.

Model	Behavioural symptoms	Predictive validity (reversal of symptom by drug)	Reference
Mice with mutations affecting ionotropic glutamate receptors			
Grin1^{hypo}, with reduced expression of NMDA GluN1 subunit	Hyperactivity Stereotypy Cognitive deficits Social interaction deficits Deficit in pre-pulse inhibition	Haloperidol Clozapine	Barkus et al., 2012a; Duncan et al., 2006; Mohn et al., 1999
Grin2a −/−, deficient in NMDA receptor GluN2A subunit	Hyperactivity Cognitive deficits Social interaction deficits Less despair-like behaviour Low anxiety	Haloperidol Risperidone	Boyce-Rustay and Holmes, 2006; Miyamoto et al., 2001
Grik2 −/−, deficient in kainate receptor GluK2 subunit	Hyperactivity Risk-taking behaviour Aggressive behaviour Less despair-like behaviour Enhanced locomotion to psychostimulant	Lithium Lithium Lithium	Shaltiel et al., 2008
Grid1 −/−, deficient in delta receptor GluD1 subunit	Hyperactivity Low anxiety Despair-like behaviour Aggressive behaviour Social interaction deficits	 Lithium D-Cycloserine	Yadav et al., 2012
Mice with mutations in proteins associated with glutamate receptors			
Shank3 overexpressing transgenic mice	Hyperactivity (in home cage) Deficit in pre-pulse inhibition Enhanced locomotion to psychostimulant Less despair-like behaviour	Valproate Valproate Valproate	Guilmatre et al., 2014; Han et al., 2013

2.9 GluA1 subunit-deficient mouse line (*Gria1*−/− mice)

Since there are no relevant subunit-selective pharmacological agents for AMPA receptors, the gene targeting approach has provided novel insights into the role of individual subunits. A mouse line deficient in *gria1 gene* (equals *GRIA1* in human genome) was generated in 1999 (Zamanillo et al., 1999) and it is available at the Jackson Laboratory (B6N.129-Gria1tm2Rsp/J, stock number: 019012). From that time, extensive research on *Gria1*−/− mice aimed at elucidating the role of the GluA1 subunit-containing AMPA receptor in learning and memory, addiction and

neuropsychiatric diseases, conditions where perturbed glutamatergic signalling has been suspected.

2.9.1 THE BEHAVIOURAL PHENOTYPE

The basic characteristics of *Gria1−/−* mice do not differ from the *Gria1*+/+ wild-type line (WT); i.e. their physical health, body weight, food consumption, nociception, neurological, motor, sexual, sensory functions and circadian rhythm seems to be normal (Bannerman et al., 2004; Chourbaji et al., 2008; Feyder et al., 2007; Fitzgerald et al., 2010; Hartmann et al., 2004; Procaccini et al., 2011; Vekovischeva et al., 2004; Vekovischeva et al., 2001).

The extensive behavioural characterization hinted at anti-anxiety, schizophrenia-like and depressive-like phenotype. In tests for anxiety-like behaviour (elevated plus maze, dark-light box), the behaviour of *Gria1−/−* mice seemed to be inconsistently altered: from somewhat less anxious (Fitzgerald et al., 2010; Vekovischeva et al., 2004), through normal (Mead et al., 2006) even to more anxious than WTs (Bannerman et al., 2004). A deficiency in pre-pulse inhibition (PPI) of acoustic startle is thought to be indicative of psychosis-related properties (Wiedholz et al., 2008). The increased learned helplessness pointed to the presence of deficits in coping abilities in aversive situations and depressive phenotype of *Gria1−/−* mice (Chourbaji et al., 2008).

Furthermore, the spatial working memory of *Gria1−/−* mice was reported to be disrupted (Reisel et al., 2002; Sanderson et al., 2009). Despite the prominent role the GluA1 has in adaptive processes underlying learning and memory, cognitive tests revealed no impairment in spatial reference memory (Zamanillo et al., 1999; Sanderson et al., 2009). In contrast, a cellular correlate for learning and memory, long-term potentiation (LTP), was lacking in Schaffer collateral - CA1 pyramidal cells of *Gria1−/−* mice, where it is believed that insertion of AMPA receptors and modifications in the GluA1 subunit are essential. Consequently, the direct linking of these two phenomena became questionable. Subsequently, spared GluA1-independent LTP of *Gria1−/−* mice was demonstrated using a different induction protocol (Hoffman et al., 2002; Jensen et al., 2003; Romberg et al., 2009). The LTP depended on GluN2B-containing NMDA receptors, neuronal nitric oxide synthase and PKC. Thus it is possible that this machinery might regulate the activity-dependent, synaptic expression of AMPA receptor by interacting with the GluA2 subunit.

As drug addiction is often described as maladaptive learning, the mouse line has been extensively utilized in studying addiction-related phenomena (Allison and Pratt, 2003; Luscher and Malenka, 2011). The development of tolerance after chronic administration of benzodiazepines and morphine is thought to involve the GluA1 subunit and it is deficient in *Gria1−/−* mice (Aitta-Aho et al., 2009; Vekovischeva et al., 2001). There is a report that the mechanism mediating withdrawal might be different in these two classes of drugs as the withdrawal after benzodiazepines was enhanced whereas that encountered after morphine was

reduced. Sensitisation to morphine was retained in *Gria1−/−* mice only in the presence of environmental cues (Vekovischeva et al., 2001). There is a report that opioid-induced glutamate receptor neuroplasticity is impaired in *Gria1−/−* mice as the morphine induced state-dependency was absent (Aitta-Aho et al., 2012).

Gria1−/− mice exhibited remarkably reduced aggression, lacking the ability to adapt to social encounters (Vekovischeva et al., 2004). In support of these data, treatment with the AMPA receptor antagonist reduced aggression in mice selectively bred for high aggression (Vekovischeva et al., 2007). Thus, it is believed that the GluA1 protein is essential for properly directing the behaviour of survival significance in the natural habitat.

The most striking and most readily reproducible phenotype of *Gria1−/−* mice is hyperactivity provoked by environment. The locomotor activity of *Gria1−/−* mice was similar to that of WT littermates in the familiar environment of their home cage (Wiedholz et al., 2008). However, locomotor activity increased dramatically (at least doubling) in an unfamiliar environment of novel cages and this elevation was sustained for up to 4-h (Bannerman et al., 2004; Chourbaji et al., 2008; Fitzgerald et al., 2010; Procaccini et al., 2011; Vekovischeva et al., 2001). In the WT mice, repeated exposure to novel cages evoked habituation, demonstrable as a diminished response, but in *Gria1−/−* mice this phenomenon was lacking. There is a report that any confrontation with a novel signal, such as a new object or littermate confrontation, may cause an aberrant reaction in *Gria1−/−* mice (Wiedholz et al., 2008).

2.9.2 THE NEUROCHEMICAL PHENOTYPE

Subsequently, other studies evaluated the neurochemical changes of *Gria1−/−* mice and found unaltered 5-HT and noradrenaline contents in various brain regions, including hippocampus (Vekovischeva et al., 2004). However, another study reported decreased hippocampal 5-HT and noradrenaline that were linked to the depressive-like phenotype (Chourbaji et al., 2008). The discrepancies in the results might be related to the different manipulations deployed, i.e. in the first experiment, the effects were analysed after a short period of social isolation but in the latter in the baseline state. Moreover, no apparent changes in dopamine transporter expression, affinity for dopamine and dopamine release appeared in striatum, but nonetheless this, dopamine clearance was slightly decreased (Wiedholz et al., 2008). The hyperactive phenotype of *Gria1−/−* mice could not be rescued simply by depletion of the dopamine level however, but it could be reversed partly by haloperidol, leading to the proposal that regulation of this behaviour is beyond dopaminergic transmission itself (Fitzgerald et al., 2010; Wiedholz et al., 2008). Indeed, in the *Gria1−/−* mouse line increased total glutamate levels have been described (Chourbaji et al., 2008), which might drive the dopaminergic system hyperactivity. The plasma corticosterone levels and hippocampal glucocorticoid receptor expression of *Gria1−/−* mice appeared to be

normal in acute stress episode, evidence for a non-altered stress-induced response in the *Gria1−/−* mice (Fitzgerald et al., 2010; Fumagalli et al., 2011).

The life-long deletion of *gria1* gene would be expected to trigger some compensatory changes, particularly when one of the key regulators of neuronal plasticity is missing. In fact, in the genome expression profiling study in hippocampal tissue, 38 genes were found to be altered by more than 30%, with the major part of those affected apparently being calcium handling genes. This is probably due to low calcium influx as the amounts of all GluA1 partnering Ca^{2+}-permeable channels were reduced. In turn this might have affected (caused elevation) one of the major regulators of Ca^{2+} inflow through the cell membrane, i.e. the GluN1 subunit protein level of NMDA receptors, as seen in hippocampus (Chourbaji et al., 2008; Zhou et al., 2009). The normal functionality of NMDA receptors (Vekovischeva et al., 2004; Vekovischeva et al., 2001) appeared to be compromised to some extent in the kind of situations that require behavioural plasticity, e.g. in stressful situations, when the serine-phosphorylation of GluN1 and GluN2B of NMDA receptor subunits was lacking (Fumagalli et al., 2011).

The activity-related changes of *Gria1−/−* neurons have been monitored with the immediate early genes (IEGs). The lack of GluA1 evoked the up-regulation of activity-regulated cytoskeletal associated protein and c-Fos in hippocampus (Fumagalli et al., 2011; Procaccini et al., 2011), in a modestly-stressful situation such as immobilisation or a change in the animal's environment. In contrast, the induction of IEGs after electroconvulsive shock remains similar between two genotypes (Zamanillo et al., 1999).

No changes were observed in synaptic transmission in *Gria1−/−* mice, however, there were extensive reductions of AMPA current in the soma of CA1 pyramidal cells in *Gria1−/−* mice (Zamanillo et al., 1999). This means that the deletion of GluA1 subunit had particularly affected extrasynaptic receptors, with the remaining GluA2/A3 and GluA3/A4 subunits being targeted to synapse. At the subcellular level, there was a re-distribution rather than any up-regulation of GluA2 subunit: it remained in the soma since there was a lack of suitable partner to achieve synaptic delivery (Mead and Stephens, 2003; Zamanillo et al., 1999). On the other hand, another study found that the levels of GluA2 and GluA4 were slightly reduced, perhaps due to a shorter half-life in the unassembled form (Jensen et al., 2003). Indeed, the functionality of remaining AMPA receptors seemed to be compromised as AMPA binding was markedly lower than the level in the WT (Vekovischeva et al., 2004). Interestingly, there were changes in the excitatory synapse to VTA DA neurons in *Gria1−/−* mice with an increase of basal AMPA/NMDA receptor current ratio being observed (Aitta-Aho et al., 2012; Dong et al., 2004), probably involving some compensatory mechanisms.

2.9.3 PHARMACOLOGICAL CHARACTERISATION

Despite being proposed as exhibiting a schizoaffective phenotype, *Gria1−/−* mice have been insufficiently characterised pharmacologically. The drugs

examined have mostly targeted monoaminergic or glutamatergic transmission and involved both acute and chronic studies. The DA D2-like receptor antagonist and neuroleptic agent, haloperidol, reduced the locomotor hyperactivity of both *Gria1−/−* mice and WT mice (Wiedholz et al., 2008). Since it is a mood-stabiliser, lithium partially rescued hyperactivity, but treatment with the inhibitor of GSK-3β, SB216763, was ineffective (Fitzgerald et al., 2010). Ultimately, no effect was seen with the positive modulator of the AMPA receptor and there were variable effects found with AMPA/kainate antagonists on the characteristic locomotor hyperactivity of *Gria1−/−* mice. Treatment with a noncompetitive antagonist of the AMPA receptors, GYKI 52466, was ineffective at attenuating the *Gria1−/−* hyperlocomotion (Fitzgerald et al., 2010), but it was dose-dependently reduced by NBQX (Procaccini et al., 2011). The competitive antagonist, NBQX, might have greater selectivity for the AMPA receptor, suggesting that transmission through AMPA receptors may be involved in mediating the hyperactive behaviour. Hyperlocomotion and reduced immobility were insensitive to manipulations of serotonergic system (Procaccini et al., 2011), which rather tend to reject the depressive-like symptomatology of the *Gria1−/−* mice as previously postulated (Chourbaji et al., 2008).

3. AIMS OF THE STUDY

The GluA1 subunit of the AMPA receptor is known to play an essential role in the several adaptive processes of our brain, important for processing of the novel signals and crucial for the reward behaviour. In addition, the importance of the glutamatergic neurotransmission in the pathophysiology of neuropsychiatric diseases has been addressed extensively. Although initially postulated as a putative model for schizoaffective symptomatology, *Gria1−/−* mice have not been subjected to an extensive substantial pharmacological characterization.

The specific aims of this study were:

1. To assess pharmacological treatments targeting glutamatergic transmission for reversing the hyperactivity phenotype of *Gria1−/−* mice in a novel environment (**I, II, III**) and to assess the role of handling on the drug effect on hyperactive phenotype of *Gria1−/−* mice (**III**).

2. To assess how pharmacological treatments targeting glutamatergic transmission could alter the behaviour of *Gria1−/−* mice in other tests specific for schizoaffective symptoms, such as anxiety-like and risk-taking behaviour, despair-like behaviour, social behaviour, hedonistic behaviour (sucrose drinking, access to running wheel) and to characterise impulsive-like behaviour of the *Gria1−/−* mice (**I**).

3. To assess the efficacy of selected pharmacological treatments on novelty-induced activation of brain regions in *Gria1−/−* mice and WTs by measuring c-Fos expression (**II, III**).

4. MATERIALS AND METHODS

4.1 ANIMALS

Gria1−/− mice and their *Gria1*+/+ wild-type controls originating from heterozygous breeding, were genotyped after weaning as reported earlier (Zamanillo et al., 1999). The *Gria1−/−* mouse line was backcrossed to C57BL/6J for more than 10 generations. During the course of the experiments, they were housed individually or in groups of 2-6 of the same-sex in plastic cages (with aspen chip beddings and a wooden toy/ shelter and nesting material), under standard laboratory conditions (12-h light-dark cycle; lights on at 6:00 a.m.; temperature 20–23ºC; relative humidity 50–60%). Food pellets and tap water were available *ad libitum.* All tests were performed between 08:00 and 14:00h. The animal testing procedures were approved by the State Provincial Government of Southern Finland (ESAVI-0010026/041003/2010 and ESAVI/2015/04.10.07/2013). All efforts were made to minimise the number and suffering of animals. Table 6 shows the total number of animals used in the studies. Male and/or female mice were randomly assigned to drug-treatment or control groups. The effect of chronic treatment with lamotrigine on locomotor activity was performed on females, whereas the assessment of behaviour in the second battery of tests in the study I (**I**, Fig.1) and in the Intellicage (unpublished data) was performed on males mainly. The tests were conducted blindly to genotype and/or treatment.

Table 6. Overview of the number of *Gria1−/−* mice and WT mice used in the studies.

Study	Test	WT		*Gria1−/−*	
		Males	Females	Males	Females
	Locomotor activity				
I, II	LI, VAL, TOP, LAM, PER	52	49	42	50
III	LY354740	49	49	47	55
I	EPM, FST	25	21	21	22
I	SD (+RW), OF, TST, SI, AMPH	36	6	20	5
	c-FOS expression				
II	LI, VAL, TOP, LAM, PER	39	38	31	37
III	LY354740	17	16	16	16
unpublished data	Intellicage				
	SD (+RW)	5		5	
	Impulsivity	17	2	25	3

LI, lithium, VAL, valproate, TOP, topiramate, LAM, lamotrigine, PET, perampanel
EPM, elevated plus maze test; FST, forced swimming test; TST, tail suspension test; SD, sucrose drinking; RW, running wheel access; OF, open field test; SI, social interaction test; AMPH, D-amphetamine-induced LA test.

4.2 DRUG-TREATMENTS

Acute or chronic drug administrations were used (Table 7). For the chronic treatments drugs were mixed with the food at the doses aimed to be equivalent to human therapeutic levels. The doses were based on those described in the literature or from pilot studies. During the course of chronic studies mice were monitored daily for all respective toxicity signs. The body weights of mice were measured at least two times weekly.

Table 7. Summary of drug-treatments.

Drug treatment	Duration	Dose/kg of chow	Administration
Lithium	Chronic	2.4 g/kg	Per os (in a chow)
Valproate	Chronic	10 g/kg	Per os (in a chow)
Topiramate	Chronic	27 mg/kg	Per os (in a chow)
Lamotrigine	Chronic	75 mg/kg	Per os (in a chow)
Perampanel	Chronic	60 mg/kg	Per os (in a chow)
LY354740	Acute	15 mg/kg 30 mg/kg	Intraperitoneal

4.3 BEHAVIOURAL METHODS

We assessed the locomotor activity, anxiety-like and exploratory behaviour, despair-like behaviour, social behaviour, hedonistic and impulsive-like behaviour. Study I utilized two behavioural test batteries to measure sets of specific behavioural symptoms. The design of testing allowed a sufficient recovery break between the tests and the order was carefully selected to minimize the bias and stress of multiple testing (**I**, Fig. 1).

4.3.1 LOCOMOTOR ACTIVITY IN A NOVEL ENVIRONMENT (I, II, III)

Locomotor activity (LA) in a novel environment was observed in plastic cages (40 × 30 × 20 cm) as described (Procaccini et al., 2011). Horizontal movements of eight to ten mice, placed in visually isolated cages in a sound-attenuated room at light intensity of 175 lx were simultaneously recorded for 2 h and analysed automatically using video tracking and EthoVision software.

4.3.1.1. HANDLING (III)

In study III, the locomotor activity was evaluated using two cohorts of mice. All mice were naïve to injections in the first cohort. In the second experiment, mice were briefly handled and injected with saline intraperitoneally daily for 1 week prior to their introduction into novel cages and measurement of LA. This was done for the purpose to analyse c-Fos expression to avoid any injection-induced stress and its influence on brain c-Fos expression.

4.3.2 ELEVATED PLUS MAZE (I)

The elevated plus maze (EPM) test was selected for the purpose of assessing innate mouse anxiety in novel unshielded places. The movements of a mouse within the central platform, open and enclosed arms of a maze were recorded for 5 min and were analysed automatically with the EthoVision software (Linden et al., 2006).

4.3.3 FORCED SWIMMING AND TAIL SUSPENSION TEST (I)

The forced swimming test (FST) as well as tail suspension test (TST) assess an animal's ability to cope in an inescapable situation by placing it in a transparent cylindrical beaker with water or attaching the mouse by the tail to an elevated metal bar for 6 min (Flaisher-Grinberg and Einat, 2009). Video-recorded data were analysed later by Ethograph software.

4.3.4 VOLUNTARY SUCROSE DRINKING AND RUNNING WHEEL ACTIVITY (I)

To assess hedonic behaviour, the preference for the sucrose and activity on running wheels (RWs) of the *Gria1−/−* mice and WTs were evaluated (Flaisher-Grinberg et al., 2009). Mice had daily alternate access to water and sucrose (8% weight/volume) for 6 days. For the next 6 days, additional natural rewarding stimulus (RW) was introduced, to test for the different interaction of the stimuli on the behaviour of *Gria1−/−* mice and WTs. As the result, the sucrose preference was calculated as a percentage of sucrose intake as a part of the total fluid. Wheel revolutions indicate running activity on RW.

4.3.5 Open field test with new object exploration (I)

Additionally, in order to evaluate anxiety and explorative activity, the open field test was combined with new object exploration (Ramos, 2008). After an initial 3 min of exploration, a round, textured object was placed in the centre of the open field and the mouse was allowed to examine it for the next 3 min. Locomotion in different zones was tracked automatically with Ethovision whereas object-related behaviours were analysed with Ethograph.

4.3.6 Social interaction (I)

Social interaction was evaluated in a new territory with fresh bedding (Vekovischeva et al., 2013). The behaviour of two to three animals was video-recorded and analysed by the Ethograph software. Their behaviour was categorized into individual behaviour (without contacts with other members), reciprocal (simultaneously-initiated) contacts, and passive contacts (initiated by other group members).

4.3.7 Response to a psychostimulant (I)

The locomotor response to a psychostimulant was tested after a single i.p injection of amphetamine and analysed by the EthoVision software.

4.3.8 Drinking behaviour as assessed in Intellicage (unpublished data)

The Intellicage system (2003, NewBehavior Lab, Zürich, Switzerland) is a computer-based, fully automated testing apparatus, designed to be placed inside a large cage (610 × 435 × 215 mm; model 2000P, Tecniplast, Buguggiate, Italy). The apparatus is constructed from four operant (drinking) triangular chambers (15 × 15 × 21 cm), each of them housing only one mouse at a time. Each chamber is constructed from an opaque tube (30 mm inner diameter) leading towards two drinking bottle nipples, left and right, which are accessible through the corresponding drinking windows (13 mm). The access to a bottle nipple is regulated by the slide-door that is easily opened by a nose-poke if the door is not specifically blocked by the computer-aided algorithm. Intellicage monitors individual drinking behaviours based on subcutaneous micro transponders that emitted a unique animal code upon entering of drinking chambers: visit(s) into the chamber, nose-poke(s) to open the slide-door, and nipple lick to drink. The

Intellicage allows comparison of the learning abilities independent of handling or introduction to new environment for testing (Galsworthy et al., 2005). The group of 4-6 mice were housed in the Intellicage under 12-h light/dark cycle (lights on at 8:00 a.m.) that was equipped with sleeping shelters and food *ad libitum.*

4.3.8.1 Assessment of voluntary drinking behaviour of *Gria1−/−* mice

Voluntary consumption was monitored under conditions of free access to every drinking chamber. The sucrose (200mM) and quinine (1mM) solutions exchanged two water bottles and were presented intermittently (every second day) for two days. For the following two days, sucrose drinking was analysed when the environment was additionally enriched with a running wheel device which the animals could use of their own free will.

4.3.8.2 Assessment of impulsive-like behaviour of *Gria1−/−* mice

We evaluated ability of mice to perform the sequential drinking protocol in a clockwise order for 3 days in the presence of water in all chambers (water conditions) or when two bottles were exchanged for sucrose and quinine solutions (sucrose-quinine conditions) (Fig. 4). In particular, the mice were allowed to drink for 5s from the correct chamber (slide-door is opened by a nose-poke), if the mouse already drank from the previous one. Thus, the mouse was forced to search for a new correct chamber on the next occasion. The violations of the protocol as the total number of incorrect visits (Vs) to chambers followed by nose-pokes (NPs) and the total number of incorrect NPs into the wrong chambers were counted for each days (using number of trials for 24-h as a covariate in the analysis). Specified mistakes in each of the chambers in the sucrose-quinine conditions were calculated as the proportion (%) of the total number of the incorrect Vs and incorrect NPs made per 24-h. The number of incorrect NPs that the mice made in the out-of-turn chamber reflected impatience of mice to get a drink from that particular chamber.

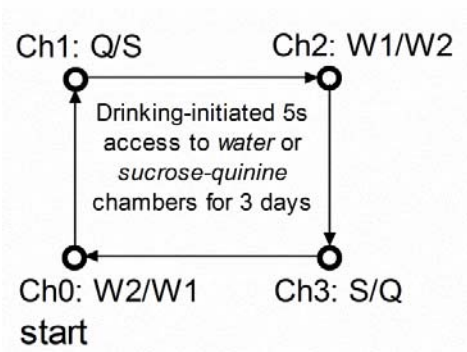

Figure 4. Experimental schedule of drinking behavior in the Intellicage under water and sucrose-quinine conditions (SQ). Bolded *circles* indicate the four drinking chambers (Ch) located in the corners of the Intellicage. *Arrows* indicate the clockwise sequence of the drinking protocol. The trial always started from the (Ch0). W1 is a water-containing chamber placed before a sucrose one, and W2 before a quinine chamber according to the clockwise order. The mice drinking behaviour in water and sucrose-quinine solutions was followed simultaneously for 3 days.

4.4 Analytical and neurochemical methods

4.4.1 Drug concentrations (I)

The concentrations of lithium and lamotrigine were analysed from the serum samples collected after test of LA in a novel environment. Specifically, the concentrations of lithium were determined by a colorimetric method in NordLab Oulu (Oulu, Finland) and that of lamotrigine by liquid chromatography after solid-phase extraction in the Rinnekoti-Foundation Laboratory (Espoo, Finland). The concentrations of other drugs used in the study could not be determined due to methodological problems.

4.4.2 c-Fos immunohistochemistry (II, III)

Following the 2-h novelty-exploration test, the brains of animals were quickly dissected, and stored at -80 °C before final cutting on a cryostat for subsequent c-Fos immunohistochemical detection. Goat anti-c-Fos antibody and biotinylated horse anti-goat secondary antibody were used, and the avidin–biotin peroxidase complex and diaminobenzidine with nickel sulphate intensification applied for visualisation. The resulting detection of c-Fos-positive cells from light-microscope captured images was automatic using ImageJ software (**II, III**), a constant

threshold and ‘region of interest’ area. The analysis was blind to the treatment, genotype and sex.

4.5 STATISTICAL ANALYSES

Statistical analyses were carried out using IBM SPSS Statistics 21 and GraphPad Prism softwares applying two- or three-way Analysis of Variance (ANOVA) or Linear Mixed Model (using genotype, drug-treatment and/or sex as factors), with repeated measures (days, time-point) and covariate, where applicable. Bonferroni, the Least Significance Difference or Newman-Keuls were used as *post-hoc* tests. Pearson’s correlation coefficients were calculated to test the significance of the correlations. Paired two-tailed *t*-test was used to compare the body weights of the mice and the differences in expression of c-Fos protein within the group. Statistical significance was set at $p<0.05$.

5. RESULTS AND DISCUSSION

Table 8 summarises the clinical symptomatology of the psychotic disorders, its putative correlates in the *Gria1−/−* mice in the representative animal tests studied here and the response to treatment targeting glutamatergic transmission, if assessed. The results of behavioural and pharmacological characterisation of the *Gria1−/−* mice will be discussed in the following chapters.

5.1 EFFECTS OF THE DRUG-TREATMENTS ON THE HYPERACTIVITY OF THE *GRIA1−/−* MICE (I,II,III)

The most robust and easily-provoked behaviour of *Gria1−/−* mice is hyperactivity, but the mechanism involved in this response has not been clarified and this can lead to biased results in other tests. For that reason, the focus of these studies was particularly on hyperactivity. It is a shared feature of several psychotic disorders, among others manic episode of BPD, SCZ and SAD (*DSM-V*). Accordingly, it was decided to probe the responsiveness of this mouse line to current pharmacotherapy in order to evaluate the predictive usefulness of the model for these disorders.

Standard mood-stabilisers, lithium and valproate (Fig. 5AB), and the novel mood-stabilising anticonvulsants, topiramate and lamotrigine (Fig. 5EF) all attenuated the aberrant activity of *Gria1−/−* mice (**I**). These drugs have been documented to possess glutamate-modulating properties and could affect components of glutamatergic synapse (Machado-Vieira et al., 2009) (Table 3). Hence control of glutamate release or the control of glutamate effects in synaptic cleft or at the postsynaptic level could exert substantial antihyperactive effects in *Gria1−/−* mice.

Furthermore, we tested the effect of LY354740, a potent and selective mGluR2/3 agonist with antipsychotic and antianxiety properties, on hyperactivity of *Gria1−/−* mice (**III**). LY354740 was able to reduce the hyperactivity of male *Gria1−/−* mice in naïve (at a dose of 15mg/kg) and pre-handled (at doses of 15mg/kg and 30 mg/kg) cohorts (Fig. 6AB). Group II mGluRs, mGluR2 and mGluR3, are located in presynaptic terminals and negatively modulate glutamate excitation by reducing its release (Marek, 2010). The use-dependent activation of these receptors provides a form of negative feedback to prevent hyperexcitability (Scanziani et al., 1997). In rodents, treatment with an mGluR2/3 agonist has been reported to inhibit PCP- and amphetamine-evoked behavioural activation (Fell et al., 2008). Since activation of these receptors attenuated the hyperlocomotion, their over-reaction to novelty could well stem from enhanced glutamatergic neurotransmission, at least in males. Accordingly, Chourbaji et al. reported higher

Table 8. Face and predictive validity of *Gria1−/−* mice for symptoms of psychotic disorders.

Clinical feature *(DSM-V)*	Animal test	***Gria1−/−*** phenotype as compared with WTs	Mood-stabilising effects on the ***Gria1−/−*** phenotype
Over-activity Psychomotor agitation (chapter 5.1)	Novelty-induced locomotor activity	Hyperactivity	Attenuation by lithium, valproate, topiramate, lamotrigine, perampanel and LY354740 (I,II,III)
Sensitivity to psychostimulants (chapter 5.1)	Amphetamine-induced hyperactivity	Exacerbated locomotor phenotype	No significant effect of drugs (I)
Risk-seeking/avoidance Increased/decreased goal-directed activity (chapter 5.2.1)	Elevated-plus maze	Increased time spent in open arms	Reduction by topiramate (I)
	Open field test combined with new object installation	Increased interaction with the new object	Alleviation after lithium and valproate (I)
		Spent more time on the centre of open field	No significant effect of drugs (I)
Resistance to despair and increased vigour or hopelessness (chapter 5.2.2)	Forced swimming test Tail suspension test	Increased swimming/ struggling	Valproate reduced struggling time (I)
Heightened (intense) sociability and social disinhibition or social withdrawal (chapter 5.2.3)	Social activity in a temporarily formed group on new territory	Frequent but short reciprocal contacts; Dominance of passive contacts	Increased time spent in social contacts with valproate (I)
Excessive involvement in pleasurable activities and seeking for reward or lack of pleasure (chapter 5.2.4)	Intermittent presentation of sweet solution in the presence/absence of running wheels	Increased preference for sucrose, but not for running wheel use	Increased sucrose consumption after lithium and valproate, but no change in running wheel activity (I)
(chapter 5.2.5.1)	Intermittent presentation of sweet and bitter solutions in the presence/absence of running wheels in Intellicage	Increased preference for sucrose in the presence of running wheels	Unpublished data (Fig. 7)
Impulsivity (chapter 5.2.5.2)	Operant drinking sequence algorithm in Intellicage, in water and sucrose/quinine conditions	Increased impatience towards sucrose-containing chamber	Unpublished data (Fig. 8-9)

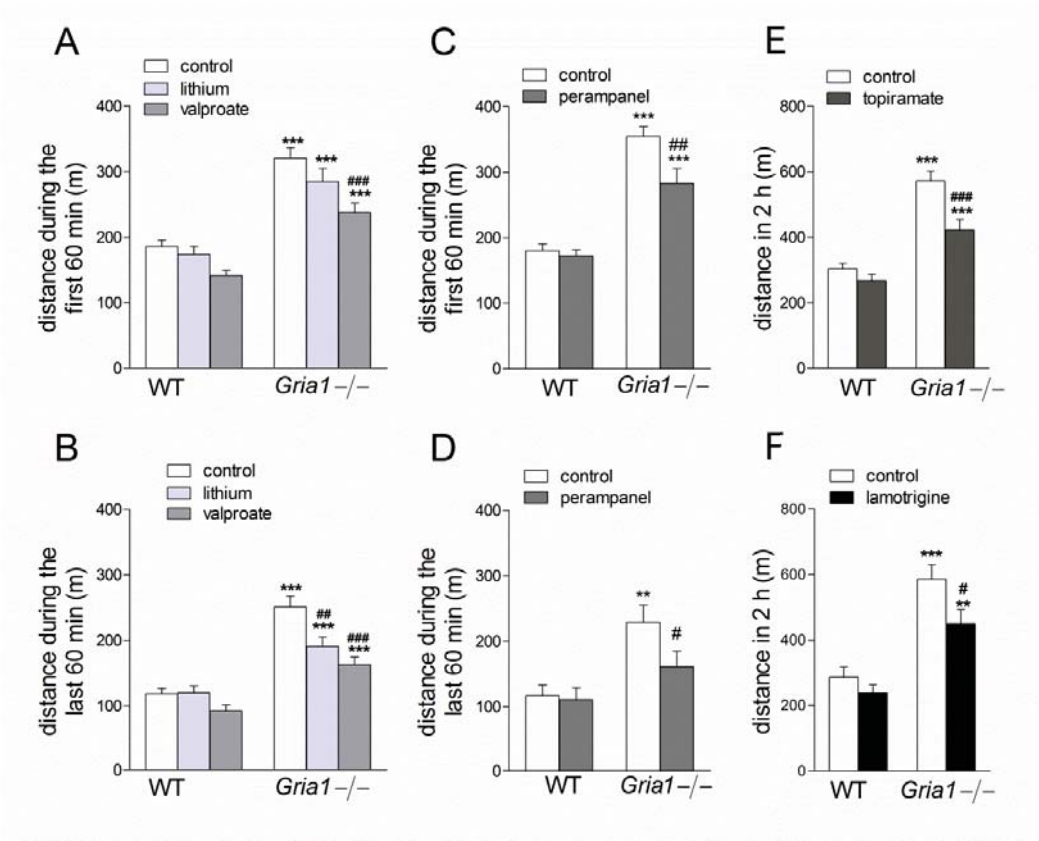

Figure 5. Effects of chronic drug-treatments on hyperactivity of *Gria1−/−* mice (I,II). Locomotor activities of WT and *Gria1−/−* mice treated chronically with control diet and lithium-, valproate- and perampanel-supplemented diets during the first and last 60 min of the exposures to a novel arena (A-D). Locomotor activities of WT and *Gria1−/−* mice treated chronically with control diet and topiramate- and lamotrigine-supplemented diets presented during the whole 2-h period (E, F). Data are means ± SEM (n = 5–27) **p<0.01, ***p<0.001 for the differences between genotypes after the same treatment; #p<0.05, ##p<0.01, ###p<0.001 for the differences from controls of the same genotype; two-way ANOVA followed by Bonferroni *post-hoc* test.

total baseline glutamate levels in hippocampus of *Gria1−/−* mice (Chourbaji et al., 2008). Given that mGluR2/3s are also present in heterosynapses, they can regulate also the release of other neurotransmitters such as GABA and monoamines (Cartmell and Schoepp, 2000). Treatment with perampanel (**II**) also attenuated the hyperactivity of the *Gria1−/−* mice (Fig. 5CD) and this finding substantiated the antihyperactive effect displayed after blockade of AMPA receptors with NBQX (Procaccini et al., 2011). Perampanel is a novel antiepileptic drug and a highly selective antagonist of AMPA receptors over other glutamate receptors (Rogawski and Hanada, 2013). These results are consistent with the presence of a hyperactive glutamatergic system and are evidence of the role of postsynaptic AMPA receptors in mediating behavioural hyperactivity in the *Gria1−/−* mice.

In fact, treatment with mood-stabilisers achieved an eventual habituation in *Gria1−/−* mice. Interestingly, the enhanced locomotion of *Gria1−/−* mice is thought to represent deficits in habituation to novel environment, congruent with defective short-term memory for spatial stimuli (Sanderson et al., 2009). However, virally-mediated introduction of GluA1 in the hippocampus of *Gria1−/−* mice abolished hyperactivity, but not the working memory deficits (Freudenberg, 2009; Freudenberg et al., 2013b) complicating direct comparisons between those matters. It appears that spatial working memory is only partially dependent on GluA1-containing AMPA receptors in hippocampus (Freudenberg et al., 2013b).

It was speculated that in SCZ primarily glutamatergic abnormalities may drive the increased responsivity of dopaminergic neurons (Lodge and Grace, 2006; Stone, 2011). Indeed, glutamatergic projections from the hippocampus increased dopamine release in the nucleus accumbens which in turn inhibited ventral pallidal GABAergic afferents to the VTA and released VTA DA neurons from the inhibition (Floresco et al., 2001; Legault et al., 2000; Lodge and Grace, 2006, 2008), with subsequently, LA being increased (Sharp et al., 1987; Taepavarapruk et al., 2000). In *Gria1−/−* mice, the VTA and striatum seem to have only a minor role in mediating the hyperactivity as these structures were not overactivated as measured by c-Fos expression in comparison to the situation in WT animals (Procaccini et al., 2011)(**II**, Table 1-3). Nonetheless, the baseline AMPA/NMDA receptor current ratio was elevated in *Gria1−/−* mice (Aitta-Aho et al., 2012). However, despite the elevation of the AMPA/NMDA receptor current ratio in the VTA DA neurons of the WT mice 24-h after the administration of diazepam, no change was detected in LA (Heikkinen et al., 2009). Similarly, the ablation of the GluA1 subunit from dopaminergic neurons does not affect LA (Engblom et al., 2008).

Amphetamine acutely exacerbated locomotion of *Gria1−/−* mice, similarly as of the WT mice (**I,** see Results). The failure of an amphetamine challenge to alleviate the hyperactivity of *Gria1−/−* mice distinguished this abnormal behaviour from the attention deficit hyperactivity disorder-type, where the psychostimulants exert paradoxical calming effects. The levels of dopamine in various brain regions of *Gria1−/−* mice in baseline and the levels evoked by amphetamine were unexpectedly normal and their striatal clearance of dopamine was only slightly reduced (Vekovischeva et al., 2004; Wiedholz et al., 2008). Thus it seems that overall, dopaminergic system itself is sensitive to the novelty or stress, but may not primarily drive the *Gria1−/−* mice abnormalities.

5.1.1 SEXUAL-DIMORPHISM ON THE ANTI-HYPERACTIVE EFFECTS OF LY354740 (III)

Intriguingly, the effect of LY354740 on attenuating the locomotor hyperactivity was restricted to male *Gria1−/−* mice (Fig.6AB) (**III**). No previous sexual dimorphism has been observed in *Gria1−/−* mouse line with regards to baseline locomotion, response to drugs or distribution of mGluR, although this may simply be due to the fact that the previous tests have been conducted only in males, or alternatively, a failure to include sex as a factor in the final analysis. Only hippocampus-mediated contextual fear-conditioning was found to differ between sexes in this line, being severely impaired in *Gria1−/−* males, but normal in *Gria1−/−* females (Dachtler et al., 2011). There are reports that the alternative GluA1-independent plasticity that relies on nitric oxide pathway can compensate for the lack of GluA1 in the hippocampus more effectively in *Gria1−/−* females (Phillips et al., 2008; Romberg et al., 2009), since neuronal nitric oxide activity is influenced by estrogen (d'Anglemont de Tassigny et al., 2009).

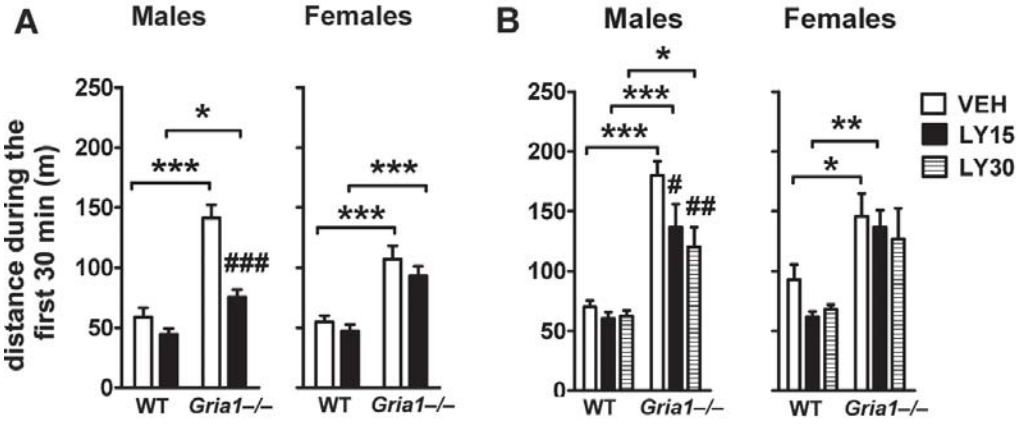

Figure 6. Effects of treatment with mGluR2/3 agonist on hyperactivity of *Gria1−/−* mice (III). Locomotor activities of naïve (A) and pre-handled (B) males and females WT and *Gria1−/−* mice after acute LY354740 (15 mg/kg (AB) and 30 mg/kg (B), i.p.) administration during the first 30 min of exposure to a novel arena. Data are means ± SEM (n = 4–19).*p< 0.05, **p<0.01, ***p< 0.001 for the difference between genotypes after the same treatment; #p<0.05, ##p<0.01, ###p< 0.001 for the difference from vehicle-treatment of the same genotype; two-way ANOVA followed by Newman–Keuls *post-hoc* test. LY, LY354740; VEH, vehicle.

Glutamate may directly activate the hypothalamic-pituitary-adrenocortical axis, which is important in adaptation to stress. Females release more corticosterone than males and they generally exhibit less anxiety-related behaviour than their male counterparts (Handa et al., 1994; Rivier, 1999); they are also less susceptible to stress-induced hippocampal plasticity. On the other hand, the administration of LY354740 itself has not been reported to alter baseline (non-stressed situation) corticosterone levels as the hypothalamus-pituitary-adrenocortical axis is predominantly under the control of group I and group III

mGluRs (Johnson et al., 2001). Given that the antihyperactive effect of LY354740 was absent in *Gria1−/−* females, both naïve and pre-handled, the sexual differences in LA after administration of this drug do not seem to be confined simply to differences in the stress responsivities between sexes.

5.1.2 Role of handling on the anti-hyperactive effect of the mGluR2/3 agonist (III)

Different levels of LA were observed after administration of mGluR2/3 agonist LY354740 in pre-handled versus naïve mice. The two-fold reduction in LA achieved by LY354740 treatment in the non-handled male *Gria1−/−* mice was markedly less evident after repeated handling (Fig.5, compare panel A and B) (**III**). It was shown that habituation to a stressor can normalize stress-induced IEG expression (Ryabinin et al., 1999) but the effect of handling on the level of corticosterone can be variable (Hurst and West, 2010; Longordo et al., 2011; Ryabinin et al., 1999). In *Gria1−/−* mice the stress-induced increase in corticosterone level was similar to that seen in the WT mice (Fitzgerald et al., 2010; Fumagalli et al., 2011); in the latter study, only male animals were used.

5.2 Effects of drug-treatments in other tests specific for schizoaffective symptoms

The *Gria1−/−* mice were also challenged on two batteries of behavioural tests over the course of the chronic treatments that allowed extensive behavioural screening for schizoaffective symptoms (**I**, Fig. 1) (Takao et al., 2007). *Gria1−/−* mice showed disinhibited risk-taking and a highly exploratory phenotype, and social deficits, which were at least partially reversible by treatment with mood-stabilisers (Table 8). Moreover, *Gria1−/−* mice exhibited a slightly higher preference for sucrose and made more impulsive choices towards sucrose. The experimental design included repeated testing and required single housing in the second test battery. It was postulated that these conditions were not primarily governing the behaviour in these tests. This assumption was based on the similar immobility times of *Gria1−/−* mice and WT in despair-like tests of mice differently housed or similar LA of mice with previous testing history and those naïve to experimentation (**I,** see Results) (Voikar et al., 2005; Voikar et al., 2004).

5.2.1 EFFECTS OF DRUG-TREATMENTS ON TESTS FOR ANXIETY-LIKE AND GOAL-DIRECTED BEHAVIOUR (I)

Results from human behavioural pattern monitoring laboratory indicate that the high mobility and alteration of exploratory behaviour are characteristics of BPD manic and SCZ patients in comparison to healthy subjects (Henry et al., 2013; Minassian et al., 2010; Perry et al., 2009; Young et al., 2007). When compared to SCZ patients, BPD manics display characteristic goal-directed activity: higher mobility with faster habituation and more interest in new objects (Perry et al., 2010). Thereafter, the behavioural disinhibition pattern has been proposed to be an endophenotype of BPD that manifests across all phases of the disorder. In the present studies, *Gria1−/−* mice undertook more center approaches and reacted more to a novel object both in the terms of frequency and time than WTs in the open field. These behaviours were sensitive to both lithium and valproate treatments (**I**, Fig.4). Additionally, a risk-taking phenotype of *Gria1−/−* mice was observed in the EPM test, i.e. *Gria1−/−* mice spent much more time in open arms, although no differences in open arm entries or the ratio of open versus closed entries were found between the genotypes (**I**, Fig.3). Treatment with topiramate reversed the risk-taking behaviour whereas valproate increased the latency of entering onto the open arms of *Gria1−/−* mice, despite the fact that all stabilisers are recognized as possessing anxiolytic properties (Corbett et al., 1991; Khan and Liberzon, 2004).

5.2.2 EFFECTS OF DRUG-TREATMENTS ON TESTS FOR DESPAIR-LIKE BEHAVIOUR (I)

FST and TST are the tests traditionally used for predicting the antidepressant-like actions of drugs (Porsolt et al., 1977) but they have also been proposed as ways of modelling the increased vigour and goal-directed behaviour of manic episode of BPD (Flaisher-Grinberg and Einat, 2009). The increased goal-directed swimming behaviour observed in the *Gria1−/−* mice in both FST and TST is in line with previous findings (Procaccini et al., 2011) and it was attenuated by treatment with valproate in the latter test (**I**, Fig.5). Unlike valproate, lithium affected WT animals by decreasing their immobility in FST (**I**, Fig.5). Both treatments markedly increased the habituation of *Gria1−/−* mice to novel environment, but they had variable effects on swimming behaviour. Thus, the valproate effect was not solely a result of reduced locomotor performance and might be reflecting the differential sensitivity of other neurotransmitter systems to drugs in this particular task. The *Gria1−/−* behaviour in TST remained insensitive to acute escitalopram injection, unlike WT animals whose immobility became markedly reduced (Procaccini et al., 2011), which might be related to differences in the functioning of the serotonergic system. The FST and TST are indeed widely used to test the drugs that affect the serotonergic system, but the levels of 5-HT of *Gria1−/−* mice and WTs in various brain regions were very similar (Vekovischeva et al., 2004). On the other hand, it

was reported that immobility time in the FST test was dependent on the level of lithium in blood (Bersudsky et al., 2007). Thus, the lithium concentration might not have reached sufficient levels for there to be any observable effect on immobility in FST, at least in *Gria1−/−* mice.

5.2.3 Effects of drug-treatments on social interaction (I)

It was found that *Gria1−/−* mice more frequently than their WT counterparts were interacting by reciprocal (simultaneously-initiated) contacts, but spent much less time engaged in the interaction. This deficit could be normalised by treatment with valproate (**I**, Fig.6). The unfamiliarity of the environment could contribute to deficits in the interaction time and frequency of social stimuli. In fact, deficits in the short-term habituation to the context of testing and altered attentional intensity might explain why *Gria1−/−* mice were unable to direct appropriately their social encounters in an unfamiliar context, but in fact they increased their social interactions after habituation to the environment (Barkus et al., 2012b). In the present case, the elevated frequency of reciprocal contact might also reflect deficits in short-term habituation to social stimuli. Thus, relative importance of both environmental and social stimuli can be competitive (Barkus et al., 2012b) or complement each other in this case and lead to the observed deficits of social contacts. It has been reported that *Gria1−/−* mice are deficient in short-term habituation processes and thus an increased attention to stimuli would even lead to enhanced long-term associations (Sanderson et al., 2009; Sanderson et al., 2010). Inappropriate associations between stimuli may be attributable to hallucinations and delusions, phenomena encountered in the SAD (Gray, 1982; Hemsley, 1994; Kapur, 2003).

5.2.4 Effects of drug-treatments on hedonistic behaviour (I)

The characteristic goal-directed and pleasure-seeking activity during manic episodes of BPD and reduced ability to experience pleasure in depression episode of BPD and in SCZ may represent abnormal reward processes. The voluntary responses of *Gria1−/−* and WT mice were compared to two different natural rewarding stimuli, sucrose and RW. Both lines of mice ran similarly in the RWs, but the *Gria1−/−* mice, irrespective of drug-treatment, consumed more sucrose than WTs during six days of sucrose presentation (**I**, Fig.7). Interestingly, in the presence of RWs, the control WT mice reduced their consumption of sucrose (**I**, Fig.7, S4 and S6 days). It was observed that rats, if given the opportunity to run, will decrease the consumption of sucrose (Satvat and Eikelboom, 2006) as might

be the case with WTs in this study. In contrast, the *Gria1−/−* mice continued to consume sucrose at a level similar to that drunk before the RWs were introduced into the cages. Thus, there was a differential sensitivity of *Gria1−/−* and WT mice to two natural rewarding stimuli, of which sucrose consumption revealed the genotype-specific difference. The mechanisms of these two stimuli seem to be different: the dopaminergic projection originating from the VTA is essential for sucrose intake (Shibata et al., 2009), whereas endocannabinoid disinhibition of VTA DA neurons has been claimed to play a role in the reward to voluntary running (Dubreucq et al., 2013). It was interesting that the AMPA/NMDA receptor current ratio in VTA DA neurons was increased in *Gria1−/−* mice (Aitta-Aho et al., 2012). Unexpectedly, treatment with standard mood-stabilisers, i.e. lithium and valproate, increased sucrose consumption in comparison to control treatment. In contrast, both drug-treatments increased running activity only in WT animals (**I**, Fig.7BD). Thus, the rewarding responses were not consistently affected by the drugs.

5.2.5 Assessment of drinking behaviour of *Gria1−/−* mice in the Intellicage (unpublished data)

5.2.5.1 Assessment of hedonistic behaviour of *Gria1−/−* mice

Free access to sucrose in the Intellicage revealed that *Gria1−/−* mice and their WTs consumed sucrose similarly on the first two days. However, *Gria1−/−* mice increased their consumption on the third and fourth days when the environment of the Intellicage became additionally enriched with RWs, whereas the WTs remained at the same level of the consumption (Fig. 7; $F_{3,43}$= 3.18, p=0.035). Both mouse lines avoided drinking quinine and the group RW activity was similar between the genotypes.

Monitoring of rodent behaviour in the Intellicage allows preservation of social settings of home cage (Galsworthy et al., 2005). On the other hand, in conventional cages if one wishes to assess the hedonistic behaviour, the mice have to be housed individually (Chapter 5.2.4). Thus, their consumption of sucrose may be influenced by the inevitable social isolation, and it has been reported that the isolation of rats can increase their sucrose consumption as compared to non-isolated rats (Hall et al., 1997). The difference in the preference for sucrose of *Gria1−/−* mice in the presence of RWs in the conventional cages and the Intellicage emphasise the importance of controlling social settings when assessing the hedonistic behaviour of mice.

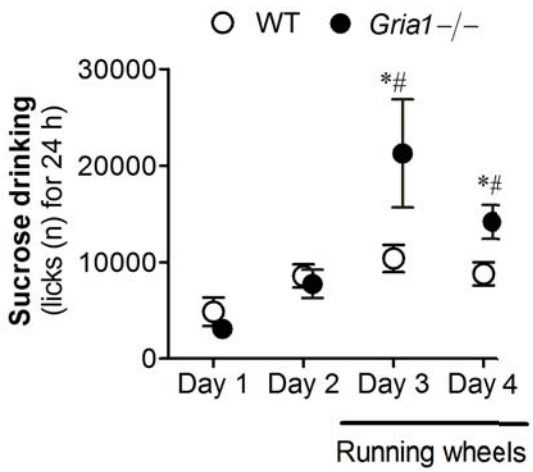

Figure 7. Hedonistic behaviour of ***Gria1−/−*** mice in the Intellicage. Voluntary sucrose consumption (number of licks) of *Gria1−/−* mice and their WTs in the Intellicage environment enriched additionally with running wheels for the last two days. Data are means ± SEM (if larger than symbol) (n=5). *p<0.05 for the difference from the corresponding value on the day 1; #p<0.05 for the difference between the genotypes; two-way repeated measures ANOVA followed by Bonferroni *post-hoc* test.

5.2.5.2 ASSESSMENT OF IMPULSIVE-LIKE BEHAVIOUR IN *GRIA1−/−* MICE

In patients with SAD, substance and alcohol abuse disorder are often comorbidities, sharing similar features such as impulsivity, novelty-seeking and disinhibition (Blanchard et al., 1999). Impulsivity may be a stable trait, but the behavioural expression of impulsivity is at its most prominent during manic or psychotic episodes (Swann et al., 2004). Impulsivity reflects poor regulation of the initiation of action, inability to wait for a reward and a deficiency in attention (Strakowski et al., 2009; Swann, 2009).

Drinking behavior was measured in the Intellicage to clarify whether the *Gria1−/−* mice would be able to perform the drinking protocol in the water and in the sucrose-quinine conditions. The total number of incorrect Vs (Fig.8A) and the total number of incorrect NPs (Fig.8B) of *Gria1−/−* and WT mice were estimated under these conditions.

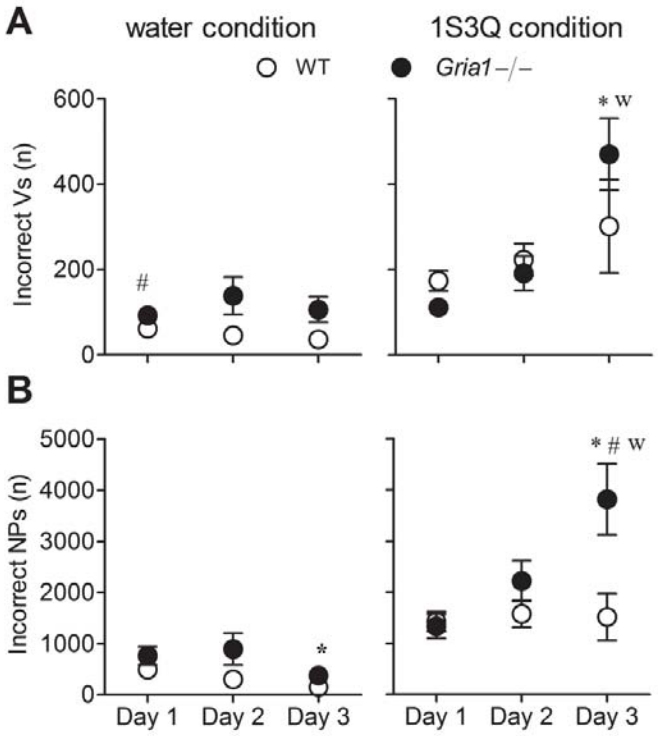

Figure 8. Drinking behavior of ***Gria1−/−*** mice during the experimental set-ups with either water or sucrose-quinine conditions in the Intellicage. The number of incorrect visits (Vs) (A) and incorrect nose-pokes (NPs) (B) are shown for each condition. Data are means ± SEM (if larger than symbol) (n=7-21). $^{\#}p<0.05$ for the difference between the genotypes; *p<0.05 for the difference from the corresponding value on the day 1; $^{W}p<0.05$ for the difference from the water condition. Linear Mixed Model followed by Bonferroni post-hoc test.

In the water conditions, the total number of incorrect Vs was higher in *Gria1−/−* mice than in WTs (Fig. 8A; $F_{1,35}=4.71$, $p<0.05$) on the first training day. However, the *Gria1−/−* mice decreased the total number of incorrect NPs on the third training day (Fig. 8B; $F_{2,35}=5.58$, $p<0.01$; using 3.42 as the average number of trials for 24-h). In the sucrose-quinine conditions, the *Gria1−/−* mice made more mistakes on the third day as compared with the first one (Fig. 8AB; $F_{2,51}=4.79$, $p<0.05$ and $F_{2,51}=3.54$, $p<0.05$ for the total number of incorrect Vs and NPs, respectively). In addition, on the third training day they made more incorrect NPs than the WTs (Fig. 8B; $F_{1,35}=5.05$, $p<0.05$; using 6.33 as the average number of trials for 24-h).

Then the specified incorrect behaviour of *Gria1−/−* mice in the sucrose-quinine conditions was evaluated in each of the chambers (Fig. 9AB). Thus, *Gria1−/−* mice made more mistakes than the WTs with respect to the sucrose-containing chamber ($F_{3,400}=6.61$, $p<0.0001$ and $F_{3,400}=6.55$, $p<0.0001$, for incorrect Vs and NPs, respectively) on the first training day ($F_{6,400}=4.35$, $p<0.0001$ and $F_{6,400}=4.38$, $p<0.0001$, for incorrect Vs and NPs, respectively), but the difference disappeared

on the other days. *Gria1−/−* mice also made fewer mistakes related with the water-containing chamber that preceded the quinine-containing chamber on the first and second training days (W2 on the Fig. 9AB). Both, *Gria1−/−* mice and the WTs made a small number of the mistakes with respect to the quinine-containing chamber during all days. Thus, the presence of the aversive quinine solution might have stimulated the mice to search for a more palatable drink and promoted impulsive behaviour in the *Gria1−/−* mice towards sucrose-containing chamber.

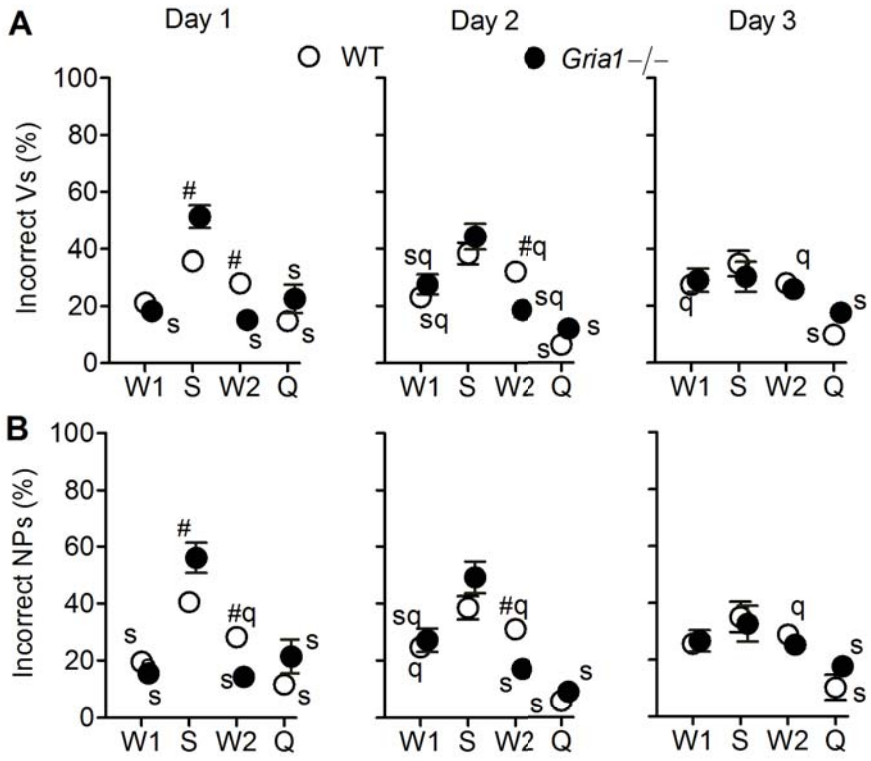

Figure 9: Drinking behaviour of ***Gria1−/−*** mice in specific chambers with respect to sucrose-quinine solutions in the Intellicage. Incorrect visits (A) and incorrect nose-pokes (B) are shown for water- (W1 and W2), quinine- (Q) and sucrose- (S) containing chambers. Data are means ± SEM (if larger than symbol) (n=14-21). $^{\#}p<0.05$ for the difference between the genotypes; $^{s}p<0.05$, $^{q}p<0.05$ for the difference between behaviour in water-chamber and behaviour in sucrose- or quinine-containing chambers, respectively. Linear Mixed Model followed by Bonferroni *post-hoc* test.

More mistakes that *Gria1−/−* mice made on the first day of the training might be related to the hyperactivity of the *Gria1−/−* mice when they are confronted with a novel situation (Fumagalli et al., 2011; Procaccini et al., 2011) and subsequent habituation to the testing context, or deficient short-term memory (Sanderson et al., 2009). In order to follow the protocol and obtain the next drink, an inhibitory response was required to reverse the learned behaviour and to switch attention to make a new nose-poke in the chamber that had been previously incorrect. This

may suggest that the *Gria1−/−* mice lack the inhibitory control and this may be comparable with the impaired pre-pulse inhibition and novelty-seeking behaviour observed previously in *Gria1−/−* mice (Wiedholz et al., 2008). Although the task was emotionally coloured (by the presence of attractive and unpleasant stimuli), this behaviour of Gria1−/− mice could not be attributed primarily to any enhanced propensity to favour consumption of sucrose, since the baseline consumption was similar in both *Gria1−/−* and WT mouse lines (Fig.6, unpublished data)(**I**, Fig.7)(Barkus et al., 2012b).

5.3 EFFECTS OF DRUG-TREATMENTS ON NOVELTY-INDUCED ACTIVATION OF BRAIN REGIONS OF *GRIA1−/−* MICE (II, III)

The baseline expression of the IEGs is very low and it rapidly increases upon stimuli that evoke neuronal activation (Procaccini et al., 2011). If one studies the patterns of IEGs expression then it is possible to create maps of the neuronal structures that underlie processing of stimuli (Kovacs, 1998). c-Fos expression was used as a cellular surrogate to map the pathways activated during aberrant locomotion of *Gria1−/−* mice.

Table 9. Summary of the effects of drug-treatments on hippocampal c-Fos expression.

Treatment	Dorsal hippocampus			Ventral hippocampus		
	DG	CA3	CA1	DG	CA3	CA1
Lithium	↔	↓	↔	↔	↔	↔
Valproate	↓	↓	↔	↓	↓	↔
Topiramate	↓	↓	↔	↓	↓	↔
Lamotrigine	↓	↓	↓	↓	↓	↔
Perampanel	↓	↓	↓	↔	↔	↔
LY354740 (in males)	↔	↔	↓	↔	↔	↔

↓ reduced, ↔ no effect

The areas activated in these studies were the cortical, hippocampal, amygdaloid and septal regions that are normally reactive to novel situations and stress (Montag-Sallaz et al., 1999). The extremely striking activation of the hippocampus of *Gria1−/−* mice (Procaccini et al., 2011) was confirmed in these c-Fos studies. The studies involved two different levels across hippocampal dorso-ventral axis in the analysis. In both *Gria1−/−* and WT mice, novelty exposure led to greater c-Fos expression in dorsal hippocampal subfields, as compared to the ventral subfields (**II,** Fig. 2-4**, III,** Fig. 3-4). This confirms the differential involvement of dorsal and ventral subfields in performance of the spatial exploratory task (Fanselow and Dong, 2010). Secondly, all glutamate-modulating

drug-treatments showed converging efficacy in blunting the excessive activation of dorsal hippocampus of *Gria1−/−* mice (Table 9) (**II,** Fig. 2-4, **III**, Fig. 3-4). Treatment with perampanel (**II**, Fig. 4) and lamotrigine (**II**, Fig. 3) reduced the number of c-Fos-positive cells in all subfields of dorsal hippocampus of *Gria1−/−* mice, whereas the other mood-stabilisers reduced the reactivity of their dentate gyrus (DG) (except lithium) and CA3 subfields (**II**, Fig. 2). LY354740 (15mg/kg) particularly reduced the reactivity of dorsal CA1 of *Gria1−/−* males (**III**, Fig. 3-4). The subfield-specific reduction of c-Fos-positive cells by drug-treatment might be related to the preferential pathways that they suppress: either direct ones, from the entorhinal cortex to each subfield, or an indirect pathway, from the DG through CA3 to CA1 (Kesner et al., 2004).

With respect to the other brain regions, only the lateral septum displayed any difference between the mouse genotypes. The response of amygdala was comparable between *Gria1−/−* mice and WT mice (**II**, Tables 1-3, **III,** Table 1) and, in fact, the characteristic 'anxiolytic-like' induction of c-Fos expression after LY354740 in the central nucleus of amygdala was lacking (**III,** Table 1). The treatment with LY354740 would be expected to suppress the excessive excitatory neurotransmission on the intervening GABAergic neurons in basolateral nucleus of amygdala, which would then restore the inhibition that the lateral part of the central nucleus of amygdala (CeL) exerts on the medial output part (Linden et al., 2005; Linden et al., 2004; Swanson et al., 2005). On the other hand, the effects of drug (as shown for amphetamine) on the c-Fos expression in the limbic regions might be precluded by the stress triggered by novel environment (Day et al., 2001; Day et al., 2005), since stress can inhibit the GABAergic neurons in the CeL.

In parallel to attenuation of hyperlocomotion in males exclusively (**III,** Fig. 2), LY354740 reduced the reactivity of CA1 subfield of their dorsal hippocampus. It was notable that the CA1 reactivity of *Gria1−/−* females to novelty as measured by c-Fos expression in the dorsal hippocampus (**III**, Fig. 4, Table 1) was lacking in contrast to the situation in their male counterparts. On the contrary, the ventral part of the lateral septum was more reactive in *Gria1−/−* females than in the males, and this phenomenon was reversed by LY354740 treatment (**III,** Table 1).

Treatment with mood-stabilisers, a selective AMPA antagonist and an mGluR2/3 agonist all blocked behavioural hyperactivity of *Gria1−/−* mice with an accompanying reduction of excessive c-Fos expression in the hippocampus. This leads to the conclusion that strong reactivity of the hippocampus is likely to account for the behavioural hyperactivity encountered in the *Gria1−/−* line and this can be attenuated by various drugs reducing glutamatergic transmission, pre- and/or post-synaptically. The hippocampus of *Gria1−/−* line is indeed vulnerable to different mild provocative situations, not only spatial novelty, as immobilisation stress has also increased expression of another IEG, activity-regulated cytoskeletal associated protein (Fumagalli et al., 2011).

Interestingly, the enhanced c-Fos expression of the dorsal hippocampus and the locomotor hyperactivity in Gria1−/− mice correlated positively (**III**, Fig. 5). c-Fos is one of IEGs and its induction is preceded by NMDA receptor activation and Ca^{2+} influx (Fleischmann et al., 2003). The global deletion of GluA1 may have caused an upregulation of Ca^{2+} binding proteins (Zhou et al., 2009) and this could be one

explanation for the excessive c-Fos activation. Interestingly, since the reference memory of *Gria1−/−* mice has been reported to be intact (Sanderson et al., 2009) and their acquisition of an instrumental stimulus response is faster (Barkus et al., 2012b), one can speculate that c-Fos could be related with their better abilities to form long-term associations and not simply be only a marker of neuronal activity (Fleischmann et al., 2003). The formation of an internal representation about a new space is a learning process and hippocampal LTP has been linked to novelty-related exploration (Li et al., 2003). Thus in the *Gria1−/−* line, the novelty exposure might promote the type of LTP that is independent of GluA1 subunit, for example as shown after theta-bursting pairing (Hoffman et al., 2002; Romberg et al., 2009).

Brain imaging studies have revealed that SCZ and BPD patients have reduced volumes of hippocampus and amygdala but enlargement of the ventricles (Arnone et al., 2009; Brambilla et al., 2001; Wright et al., 2000), although conflicting results exist in the latter case. The promotion of neuronal proliferation might contribute to the effects of antidepressants and mood-stabilisers, given the characteristic delay before the appearance of their therapeutic effects. Lithium, valproate and lamotrigine all have positive effects on neurogenesis (Manji et al., 2000; Leng et al., 2013) and are of great interest in various models of neurodegenerative disease due to their neuroprotective and neurotrophic actions (Alvarez et al., 1999; Chiu et al., 2013). There are reports that the *Gria1−/−* mice exhibit decreased neuronal proliferation but increased survival of new neurons (Procaccini et al., 2011) in the adult hippocampal DG. The chronic treatments in the study might have differentially affected hippocampal neurogenesis and functional integration of new hippocampal neurons in the *Gria1−/−* mice. Given the functional link between hippocampal neurogenesis and novelty-induced hyperactivity (Lemaire et al., 1999), a restoration of hippocampal circuitry in the *Gria1−/−* mice would result in better habituation to a novel environment. Interestingly, c-Fos, has also been shown to regulate the expression of neurotrophic factors, and these could promote neuronal survival after excessive stimulation and protect neurons from excitotoxicity (Zhang et al., 2002).

5.4 GENERAL DISCUSSION

5.4.1 COMPARISON OF *GRIA1−/−* MODEL WITH OTHER MOUSE MODELS OF RELEVANCE TO SCHIZOAFFECTIVE SYMPTOMATOLOGY

The comparison of the *Gria1−/−* phenotype with other genetic animal models of relevance to schizoaffective symptomatology (Table 5) is summarised below (Table 10). It is notable that the hyperactivity is the most commonly reported characteristic in these models and, as discussed, is the hallmark of psychotic disorders (Perry et al., 2010; Young et al., 2007).

The antagonism of NMDA receptors produces positive symptoms such as psychomotor agitation, negative symptoms such as anhedonia and social withdrawal, as well as cognitive symptoms. Similarly, the deficits observed in the *Grin1*^{hypo} mice, with the reduced expression of the GluN1 subunit of NMDA receptors, are wide-ranging, including profound impairments in several cognitive tasks but may not mimic selectively schizoaffective phenotype (Table 5, 10)(Barkus et al., 2012a). Instead, preferential deletion of GluN1 on cortical and hippocampal parvalbumin-positive interneurons (Belforte et al., 2010) but not on excitatory neurons in layer II/III of the medial prefrontal cortex and sensory cortices (Rompala et al., 2013) may represent a more comprehensive model. The *Grin2a−/−* mice share some features with the SAD (Miyamoto et al., 2001), but there are some conflicting reports (see Boyce-Rustay and Holmes, 2006)(Table 5, 10). For example, they do not exhibit deficits in pre-pulse inhibition as it has been claimed that both GluNR2A- and GluNR2B-containing receptors need to be affected for their disruption of this feature (Spooren et al., 2004). Kainate receptors are extensively expressed in hippocampus and are important for the control of inhibitory and excitatory transmission (Bureau et al., 1999; Fisahn et al., 2004). *Grik2−/−* mice display behavioural alterations that mimic some of the behavioural symptoms of manic episode of BPD (Table 5, 10). In *Grid1−/−* mice, the co-occurrence of both mania- and depression- like behaviours suggests that these mice may have some face validity for bipolar-like behaviour (Table 5, 10). Moreover, the deletion of GluD1 evoked changes in the expression of GluA1, GluK2 and glutamic acid decarboxylase 67 in amygdala and prefrontal cortex, which suggests that both excitatory and inhibitory synaptic neurotransmission are affected. The mice overexpressing *Shank3* scaffolding protein at the PSD have been reported to display manic-like symptoms, seizures and increased hyperexcitability discharges on electroencephalogram (Han et al., 2013). Indeed, in *Gria1−/−* mice some positive symptoms can be reversed by blockade of AMPA receptor with the antiepileptic perampanel and two other primarily antiepileptic drugs, valproate and topiramate. Together with aforementioned models, this supports the concept that there is an imbalance between excitatory and inhibitory transmission in favour of excitation in the pathogenesis of seizures (Magloczky and Freund, 2005) and a broad spectrum of neuropsychiatric disorders (Lisman et

al., 2008). It is now evident that epileptic and schizoaffective symptoms share many overlapping epidemiological, clinical, neuropathological and neuroimaging features (Cascella et al., 2009).

Table 10. Comparison of behavioural phenotypes between *Gria1−/−* mice and previously described mouse models of schizoaffective symptomatology.

Behavioural symptom	*Grin1^{hypo}*	***Grin2a−/−***	***Grik2−/−***	***Grid1 −/−***	*Shank3 Tg*	***Gria1−/−***
Open field hyperactivity	Yes	Yes	Yes	Yes	Yes	Yes
Home cage hyperactivity	Nt	No	Yes	Nt	Yes	No
Less anxious or more risk-taking behaviour	Nt	Yes	Yes	Yes	Nt	Yes
More aggressive behaviour	Nt	Nt	Yes	Yes	Nt	No
Deficient social interaction	Yes	Yes	Yes	Yes	Yes	Yes
Increased locomotion to amphetamine	Nt	Nt	Yes	Nt	Yes	No
Less despair-like behaviour	Nt	Yes	Yes	No	Yes	Yes
Increased reward-seeking behaviour	Nt	Nt	Nt	Nt	Nt	Yes
Deficient pre-pulse inhibition	Yes	No	Nt	Nt	Yes	Yes
Cognitive deficits	Yes	Yes	Nt	Nt	Nt	Yes
Impulsive-like behaviour	Nt	Nt	Nt	Nt	Nt	Yes
References	Barkus et al., 2012a; Duncan et al., 2006; Mohn et al., 1999	Boyce-Rustay and Holmes, 2006; Miyamoto et al., 2001	Shaltiel et al., 2008	Yadav et al., 2012	Guilmatre et al., 2014; Han et al., 2013	Bannerman et al., 2004; Fitzgerald et al., 2010; Procaccini et al., 2011; Sanderson et al., 2009; Vekovischeva et al., 2004; Vekovischeva et al., 2001; Wiedholz et al., 2008; Zamanillo et al., 1999; Study I, III; unpublished data

Not tested (Nt)

There is a glutaminase-deficient mouse line which has reduced glutamate and elevated glutamine levels in the brain, and these animals exhibit hippocampal hypoactivity in imaging and electrophysiological studies, and the opposite phenotype as seen with schizoaffective-like models (Gaisler-Salomon et al., 2009). These data corroborate the present finding that there is a specific dysfunction in the hippocampi of *Gria1−/−* mice which contributes to behavioural abnormalities.

The presentation of novel environment induced activity in the hippocampus in both WT and G*ria1−/−* mice, but the latter animals reacted far more robustly. It is evident that the hippocampus acts as a comparator of inputs and stored information and triggers novelty-dependent firing of the VTA via polysynaptic pathway (Grace et al., 2007; Lisman and Grace, 2005). The VTA DA neurons combine novelty signals with information about the relative importance of stimuli and goals (through the limbic inputs of pedunculopontine tegmental nuclei projecting to VTA)**.** The dopaminergic cells of the VTA project back to the hippocampus and induce dopamine release that facilitate the plasticity at the CA3 inputs to CA1, and thus the entry of behaviourally significant information into long-term memory (Bernabeu et al., 1997). On the other hand, a hyperdopaminergic state (through DA effects on D1 and D2 DA receptors) can selectively inhibit the continuing sensory inputs to CA1 from entorhinal cortex. A mismatch system of the CA1, a putative comparator of arriving sensory information from cortex and predictions from CA3, is disrupted and the whole loop is further enhanced (Lisman and Otmakhova, 2001). Thus the hippocampal – VTA loop in G*ria1−/−* mice may be dysfunctional**,** and G*ria1−/−* mice may be unable to filter sensory stimuli and habituate. Thus they would persistently perceive stimuli as novel and inappropriately attribute them with salience (Kapur, 2003). This may be related to abnormalities with the inhibition and attention, as the deficient PPI, working memory problems, impulsivity and enhanced reference memory are observed in G*ria1−/−* mice. Furthermore, the VTA AMPA/NMDA receptor current ratio in G*ria1−/−* mice appeared to be increased (Aitta-Aho et al., 2012).

The complexity of symptomatology, polygenic nature and the lack of objective biomarkers prevent the modelling of neuropsychiatric diseases in rodents, but nonetheless the use of genetic rodent models is indispensable in clarifying the contribution of a gene to a characteristic phenotypic expression. It has been found that the hyperactivity appeared concomitantly with other behavioural abnormalities indicative of highly disinhibited behaviour of *Gria1−/−* mouse line. The co-occurrence of these behavioural abnormalities in *Gria1−/−* mice that are controlled by standard and investigational mood-stabilisers suggests that the lack of GluA1 protein all over the brain and in different stages of the development may be involved in the pathophysiological changes underlying schizoaffective symptomatology. The *Gria1−/−* model may provide the evidence for the interaction between the glutamatergic and dopaminergic systems in the schizoaffective phenotype. It is noteworthy that aggressiveness as a correlate of irritability and easily provoked behaviour is not present in *Gria1−/−* mice (Vekovischeva et al., 2004).

5.4.2 COMPARISON OF GLOBAL *GRIA1−/−* MODEL WITH THE CONDITIONAL *GRIA1−/−* MODELS

Deletion of GluA1 in conventional *Gria1−/−* mice is not cell-type specific, which means that its absence must have affected both main excitatory and inhibitory

neurotransmitter systems. In this respect, the complexity of the conventional phenotype may be the result of the large non-selective deletion, together with subsequent compensatory changes. Since the GluA1 subunit is particularly abundant on inhibitory GABAergic neurons (Catania et al., 1998; Leranth et al., 1996), it was speculated that the inability of *Gria1−/−* mice to activate NMDA-mediated responses on interneurons would reduce GABAergic tone, disinhibit neurons and lead to abnormal neuronal activation (Fumagalli et al., 2011). The contribution of these systems and particular brain areas in driving specific abnormalities in *Gria1−/−* mice have been recently addressed at the network and behavioural levels.

A mouse line with the temporal and site (cell)-specific ablation of GluA1 subunit has been created thus ablating the GluA1-containing receptors from principal (glutamatergic) neurons under the control of the CaM kinase II alpha promoter during late adolescence, the vulnerable stage for the onset of most of neuropsychiatric disorders (Inta et al., 2013). The behavioural deficits exhibited by this mouse line include cognitive impairments, sensorimotor gating deficits and marked hyperlocomotion without the alterations of the levels of DA in striatum. Therefore, deletion of GluA1 subunit-containing AMPA receptor from principal neurons but with a spared subunit in GABAergic interneurons, phenocopies all of the deficits seen in mice with global reduction of GluA1, except that this alteration leaves sociability intact. There are reports that this deletion particularly affected the hippocampus, and induced a similar subcellular redistribution of GluA2 and upregulation of GluN2A and GluN2B as in *Gria1−/−* mice (Inta et al., 2013; Zamanillo et al., 1999).

The congruent behavioural phenotypes of mouse-lines with inducible and conventional deletion of GluA1 confirmed the vulnerability of the hippocampus. The important conclusions of these studies are as follows: (1) a hippocampal GluA1 deficiency exhibits an important schizoaffective-like phenotype and that (2) the existence of a GluA1 deficiency on glutamatergic neurons has correlates to schizoaffective symptomatology, whereas its deficiency on GABAergic neurons may relate to depressive symptomatology (Vogt et al., 2014). Thus, the forebrain GluA1 ablation model was useful for differentiating contribution of glutamatergic and GABAergic neurotransmitter systems with regard to mood and psychotic disorders as well.

Interestingly, the model with the spatially restricted GluA1 deletion shows that hyperactivity persists despite the presence of intact GABAergic tone, and points to a major role of principal neurons of the hippocampus in triggering locomotor hyperactivity. In fact, virally-mediated introduction of GluA1 in the dorsal or complete hippocampus (to both principal and interneurons) was reported to abolish hyperactivity of *Gria1−/−* mice (Freudenberg, 2009). In support of this view, mice lacking GluA1 only in forebrain interneurons show WT-like LA (Fuchs et al., 2007) but, in contrast, *Gria1−/−* mice expressing ^{GFP}GluA1 in principal forebrain neurons remained hyperactive (Freudenberg et al., 2013a).

In summary, it seems likely that hippocampal AMPA dysfunction is a major contributor to behavioural hyperactivity of *GluA1-/-* mice. This is supported with

results of the present (**III,** Fig. 4) and a previous study of hyperactivity-attenuating effect by AMPA antagonists (Procaccini et al., 2011).

6. SUMMARY AND CONCLUSIONS

1. The hyperactive phenotype of *Gria1−/−* mice can be attenuated by targeting glutamatergic transmission. Two standard antiepileptic drugs, lithium and valproate, and the novel mood-stabilising anticonvulsants, topiramate and lamotrigine, all attenuated aberrant activity of *Gria1−/−* mice. Furthermore, the putative antipsychotic, mGluR2/3 agonist LY354740, and antiepileptic, the selective AMPA antagonist, perampanel demonstrated the same effect. Thus the control of glutamate release or the control of glutamate effects in the synaptic cleft or at the postsynaptic level appear to exert substantial antihyperactive effects in *Gria1−/−* mice. The *Gria1−/−* mice seem to represent a model for the screening of the effects of novel drugs on the hyperactive behaviour relevant to psychotic disorders.

2. *Gria1−/−* mice displayed disinhibited risk-taking, less-despair like and highly exploratory phenotype as well as social deficits, which were at least partially reversible with mood-stabilisers. *Gria1−/−* mice exhibited a slightly higher preference for sucrose and made more impulsive choices towards sucrose. These tests are of particular relevance for other symptoms of psychotic disorders and they can be considered as important complements to locomotor activity test in assessments of drug efficacy in *Gria1−/−* mice.

3. All glutamate-modulating drug-treatments showed converging efficacy in blunting the excessive activation of the dorsal hippocampus of *Gria1−/−* mice, as measured by c-Fos expression. The motor activity of *Gria1−/−* mice correlated with dorsal hippocampal c-Fos expression. The highly-reactive hippocampus of *Gria1−/−* mice may account for behavioural hyperactivity. c-Fos is a sensitive molecular marker and it may prove to be a useful parameter in addition to behavioural screening evaluating for novel drug efficacy in *Gria1−/−* mice.

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Milica

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9. Original publications